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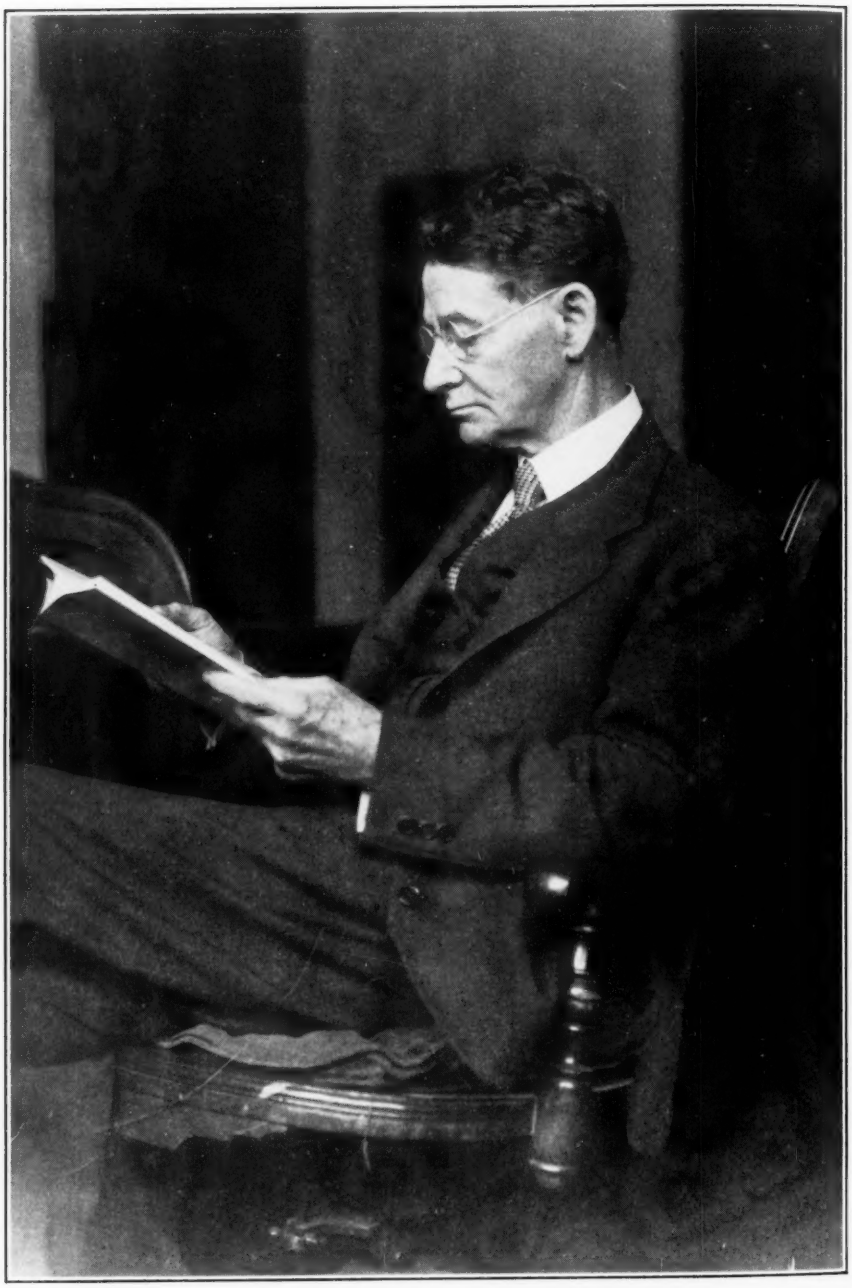

GEORGE PERKINS CLINTON
(1867–1937)

GEORGE PERKINS CLINTON
(1867–1937)

FLORENCE A. MCCORMICK

After serving thirty-five years as Botanist of the Connecticut Experiment Station Dr. George Perkins Clinton retired from active service July 1, 1937, but he was retained on the Station staff as Consulting Botanist. While his retirement was voluntary and desired on his part, no doubt the giving up of active duties was difficult, for each morning of the short time that remained he was at his desk pursuing his usual routine. On July thirteenth he became ill and on August thirteenth he passed away. His widow, two sisters, and a brother survive. A son, the only child, was killed in France during the Great War.

Dr. Clinton was born at Polo, Illinois, May 7, 1867; but his ancestors came from Massachusetts, Connecticut, and New York. A grandfather, accompanied by his sister and their families, went west in covered wagons. Possibly as a precaution against loss of his money on the way he had invested in broadcloth which was sold to the settlers in Illinois where he established a new home. This grandfather fought in the War of 1812 and two great grandfathers served in the American Revolution.

As a child, Dr. Clinton lived the normal life of an active boy, enjoying the freedom of a small town, and having the family cow, horse, chickens, and wood-pile to give him outdoor occupation. For nearly fifty years his father was editor of the Ogle County Press, and in the newspaper rooms the son was given small tasks. There also he learned much about the process of printing and about editorial work that later was of value. In the home there was a wealth of books and magazines for old and young. In school he was studious and eager to do his best, but he was modest in accepting honors. Spelling was the one subject difficult and distasteful to him, but his determination to conquer is shown by his habit of studying that particular subject with clenched fists. From his mother he inherited his great energy. The habit of collecting may have come from the example set by his father who collected coins, stamps, and geological specimens in his earlier days and books and letters throughout his life. Probably his father's great love for flowers started the boy on his botanical career.

Dr. Clinton was graduated from the Polo High School in 1886. By this time he had already become interested in botany and through a mutual friend he met Dr. M. B. Waite who encouraged him in his decision to attend the University of Illinois and to study under Dr. T. J. Burrill. He received the degree of Bachelor of Science in 1890 and was then appointed Assistant Botanist in the Agricultural Experiment Station of the University of Illinois and Assistant in Botany in the University. His quiet enthusiasm for botany made an impression upon his associates. In 1894 he received the degree of

Master of Science from the same University. During his fourteen years of residence there, many interesting changes occurred. He entered the University before there was an Experiment Station in the State. He saw the preparations for its organization and its final establishment. At that period a botanist through training and official position was interested in all phases of the subject. Thus through his relationship with Dr. Burrill, Dr. Clinton received a breadth of training that is often lacking at the present time. With the establishment of an experiment station, parasitic fungi naturally received increased attention and Dr. Clinton became especially interested in the Ustilaginales and the Uredinales. He had published papers on both these groups while a student at the University.

During this time he began a correspondence with Professor Farlow for whom he had the highest regard in a personal, as well as in a professional way. With two friends as companions, he went from Illinois to Cambridge, Massachusetts, by bicycle, carrying with him a fresh suit in which to pay his respects to Professor Farlow. In 1900, he enrolled as a graduate student at Harvard. From that University he received the degree of Master of Science in 1901 and that of Doctor of Science in 1902.

While at Harvard, Dr. Clinton still retained his positions at the University of Illinois, but he was released from his obligations there to become Botanist at the Connecticut Agricultural Experiment Station on July 1, 1902. This position was formerly held by Professor Thaxter, the first Botanist of the Station and one of Dr. Clinton's professors at Harvard. The devotion, a kind of reverent devotion mingled with idealism, which Dr. Clinton gave to Professor Farlow, he also gave to Professor Thaxter. He had a quiet pride in occupying the position once held by the man whom he so much admired. All things at the Station that had been connected with Professor Thaxter were most carefully cherished. The old desk and most uncomfortable desk chair were retained with the conclusive explanation that they had been used by Thaxter. On the scientific side, the original potato scab culture, the old wash-boiler spray outfit, devised and used by Thaxter in 1888, and some fine old drawings were among his highly prized treasures of the department. Dr. Clinton was also greatly interested in the life and poems of Cecilia Thaxter, the mother of Professor Thaxter. In 1936, he and Mrs. Clinton visited the Isle of Shoals where she had lived and is buried; and he had planned a second visit there.

Upon coming to Connecticut, Dr. Clinton entered into the agricultural activities of the State. He took a personal interest in the problems of the farmers and preferred to carry out his experiments for the control of a disease on the land where the disease was found. By that method he also came into closer contact with the grower and many of his field experiments were carried on in the days when slow and inconvenient means of travel were used by Station workers. Potato and tobacco early received his attention. In his study of *Phytophthora infestans*, he was the first to produce its oo-

spores in culture. His interest in that fungus never decreased and, only a few days before he was stricken, he spent an entire, very hot day in potato fields looking for the first appearance of late blight. This exertion probably hastened the fatal attack. He thought there was a possible connection between the potato and tomato in the transmission of the fungus and with this in mind he studied the proximity of the two crops in the field. Thus one phase of the problem, which he tried so hard to clarify, was left unsolved. He was tireless in his efforts to show growers the necessity of careful sanitation in preventing the spread of mosaic of tobacco and of the treatment of the soil of seedbed and field in combating black root rot. Years later he clearly demonstrated that the main infection of tobacco with the wild-fire organism occurs in the seedbed, which must be carefully watched and sprayed. The chestnut blight, white-pine blister rust, willow scab, and the Dutch elm disease are the major tree problems that he studied. Of outstanding importance is his work on smuts, begun at the University of Illinois and continued at Harvard. The results of this investigation are included in his Monograph of North American Ustilagineae published by the Boston Society of Natural History, and in his Ustilaginales issued in the North American Flora series. An extensive bibliography and a personal study of type specimens, as far as he was able to obtain them, and of his own collection are embodied in these publications. In the Station herbarium, the large collection of smuts, including specimens received from botanists all over the world and also specimens collected by him, testifies to the comprehensiveness of this work. Second to his interest in smuts was his general interest in rusts; and of these also there is a large collection in the Station herbarium. The department library and herbarium, housed in the same room, stand as a memorial to Dr. Clinton's industry, foresight and zeal in collecting, and are an outstanding asset of the Department as well as of the Station. The room with its excellent arrangement was planned by him. The foreign and domestic periodicals, the old and rare books, the many volumes of exsiccati, and the large collection of specimens, chiefly of fungi collected by him, were almost wholly assembled during his years at the Station. He also made a complete catalogue of all fungi mentioned in his publications. In 1935 he formally gave to the Station all his botanical books, reprints, bulletins, lantern slides, specimens, and letters from botanists all over the world. Among the letters from botanists there are interesting acknowledgments of the identification by him of hundreds of specimens of fungi.

Dr. Clinton was Botanist of the Connecticut State Board of Agriculture from 1902 until 1925, when the work of that Board was taken over by the Connecticut Department of Agriculture. He was chairman of the Committee on fungous diseases of the Connecticut Pomological Society from 1903 to 1927. A charter member of the Connecticut Asparagus Growers' Association organized in 1912, he also gave his support to that group. In the following year, when that organization became the Connecticut Vegetable Growers'

Association, he was one of the five subscribers for its incorporation. From 1919 until his death he was one of the three members of the Connecticut Tree Protection Examining Board, the first of its kind in this country. Through these diverse contacts he kept in touch with all phases of agricultural problems.

In 1904, at the request of the United State Department of Agriculture, Dr. Clinton went to Puerto Rico to study diseases of coffee. In 1908 he was called to Harvard to study fungous parasites of the brown-tail moth. The following year Harvard sent him to Japan to collect and bring back parasites for controlling the gypsy moth. In 1912 he went south to study the relationship of the chestnut blight fungus to other fungi found there. While on these missions, as well as on three trips to Europe, he collected fungi. In fact, wherever he went, for pleasure or for business, he was always collecting. From 1915 to 1926 Dr. Clinton was lecturer in forest pathology in Yale, where he was also research associate in botany from 1926 to 1929. This connection gave him an opportunity to associate with young men, in whom he was greatly interested. He was tireless in his efforts to bring the best to his students and he thoroughly enjoyed the collecting trips with his classes. His interest in his students was not limited to their work in the classroom; and they frequently came to him for consultation and advice in personal affairs.

Dr. Clinton was a fellow of the American Association for the Advancement of Science and a sustaining life member of The American Phytopathological Society, of which society he was president in 1912. He was a member of the Mycological Society of America, American Society of Plant Physiologists, Botanical Society of America, New England Botanical Society, Connecticut Botanical Society, Society of American Naturalists, Connecticut Vegetable Growers' Association, Connecticut Pomological Society, Connecticut Forestry Association, and Sigma Xi. He was made a fellow of the American Academy of Arts and Sciences in 1914 and a member of the National Academy of Sciences in 1930. In 1935 Connecticut State College gave him "Honorary Recognition" as a leader in agriculture and rural life.

As a man, Dr. Clinton has been described by his sister as "sincere, honest, generous, loyal, democratic, determined, and just." His associates also found in him all these traits in a very high degree, and to these may be added a keen sense of humor and an intense hatred for pretense of any kind. Those in sorrow caught glimpses of an unusual sympathy and his deeply religious nature. The colored man of all work who visited him during his illness and wept bitterly beside his casket remembered how often Dr. Clinton had helped him throughout the years of depression. He was comforted in his sorrow when he was asked to carry out some of the flowers and to help put back the house into its former order. The caretaker of the Station greenhouse did not want to attend the services because he felt so keenly the loss of a friend. These are tributes which testify to an innate kindness which was often con-

cealed. His frank and fearless expression of opinion were at times misunderstood but now are greatly missed and more fully appreciated.

Grateful acknowledgments are given to Mrs. Clinton; Miss Lucile Clinton, a sister; Miss Emma R. Pearson, one of his former teachers at Polo; and Dr. M. B. Waite for items about Dr. Clinton's life; to Dr. A. A. Dunlap for the photograph, which he took June 30, 1937; to Dr. Perley Spaulding for the use of his card catalogue and to the librarians of Harvard and the University of Illinois for checking over the bibliography.

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DISPERSION AND GROWTH OF BACTERIAL CELLS SUSPENDED IN AGAR

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The poured-plate technique described by Koch (9) in 1883 was the first method devised for obtaining pure cultures of bacteria from single cells. Although most investigators have accepted Koch's conclusion that each colony probably develops from a single isolated cell, other workers claim that the poured plate is not a reliable means of obtaining single-cell cultures and that pure cultures should, therefore, always be obtained by the isolation of single cells under microscopic control. Some advocates (3) of the latter method agree with Koch's conclusions but object to his method, because it furnishes no visual evidence that any particular colony developed from only one cell. Others (12, 13) believe that colonies of single-cell origin probably never are obtained in poured plates, because bacterial cells are held together by slime and because melted agar causes them to agglutinate. It even has been suggested (19) that if single cells were isolated in agar they would not grow, because single cells secured by micromanipulation usually fail to grow when placed in large quantities of broth.

Apparently, neither those who rely upon the poured plate nor those who question its reliability have tested its efficiency by direct experimentation. Studies, therefore, were undertaken to determine what proportion of the colonies of *Phytomonas stewarti* (E.F.S.) Bergey *et al.* that developed in poured plates were of single-cell origin, what effect different techniques of

pouring the plates had on the dispersion of cells and, finally, why relatively few colonies developed on dilution plates seeded with many bacterial cells. In order to supplement the data on *P. stewarti*, somewhat less extensive studies were made on the following phytopathogenic bacteria:[1] *Erwinia carotovora* (Jones) Hol., *P. campestris* (Pam.) Bergey *et al.*, *P. phaseoli* (E.F.S.) Bergey *et al.*, *P. angulata* (F. and M.) Bergey *et al.*, *P. savastanoi* (E.F.S.) Bergey *et al.*, *P. pisi* (Sack.) Bergey *et al.*, *P. tabaca* (W. and F.) Bergey *et al.*, *P. pruni* (E.F.S.) Bergey *et al.*, *P. translucens* var. *undulosa* (E.F.S., J. and R.), *P. tumefaciens* (E.F.S. and Town.) Bergey *et al.*, *P. juglandis* (Pier.) Bergey *et al.*, *P. insidiosa* (McC.) Bergey *et al.*, *P. michiganensis* (E.F.S.) Bergey *et al.*, and *P. fascians* Tilf. Data on the dispersion and growth of the different species in agar are presented in this paper.

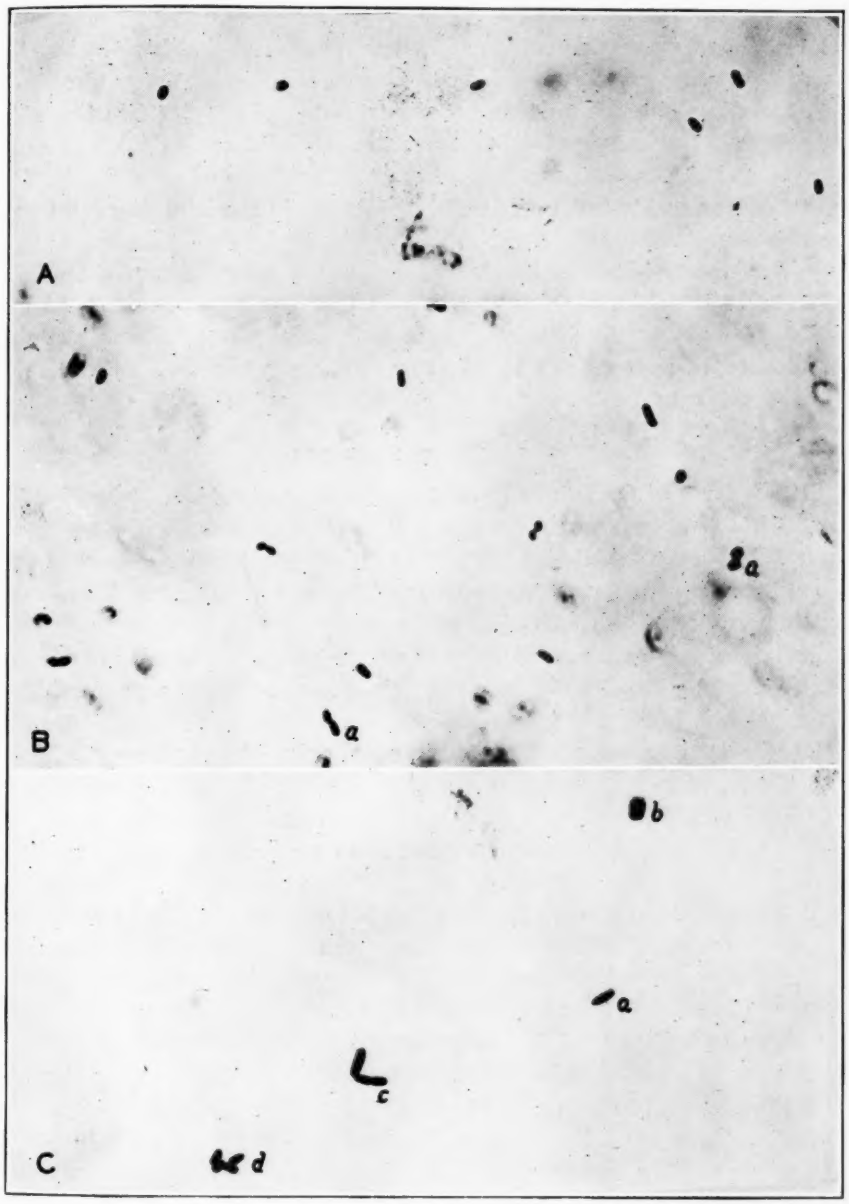
MATERIALS AND METHODS

The cultures used had been isolated for periods of from 1 to 3 years. Their identity was confirmed by infection of appropriate hosts. All species were typical (4) in regard to general cultural characteristics on nutrient agar and in nutrient broth except *Phytomonas fascians* and *P. savastanoi* which were of the rough-colony type. The strains of *P. stewarti* used were derived from culture B–11 described elsewhere (10). These strains differed in virulence, but all of them were typical in colony appearance except A–17, which produced dark yellow, semi-rough colonies. This culture was isolated from an inoculated plant grown in sand and deprived of nitrogen. The other strains had been derived as single-colony isolates from pure cultures.

Nutrient broth (Difco) supplemented with 0.5 per cent dextrose and adjusted to pH 6.8 to 7.0 was used in all experiments except where otherwise designated. The synthetic medium contained 0.858 g. of KH_2PO_4, 0.684 g. of $Ca(NO_3)_2 \cdot 4H_2O$, 1.75 g. of $MgSO_4 \cdot 7H_2O$, 0.37 g. of $(NH_4)_2SO_4$ and 15 g. of dextrose per liter. The ingredients were mixed and adjusted to pH 6.9 before sterilizing. All agar preparations contained 1.5 per cent of air-dry, shredded agar except where otherwise designated.

Suspensions of bacteria in agar were prepared for microscopic examination as follows. A 12- to 16-hour-old broth culture, presumably in the logarithmic phase of growth (1), was used in order to secure a high percentage of viable cells. The culture was thoroughly agitated, diluted in broth, agitated again, and then dispensed into test tubes containing melted nutrient agar at approximately 42° C. A concentration of bacteria about 1000 times that ordinarily used in poured plates was employed to facilitate counting. After the agar hardened it was sliced about 0.5 mm. thick with a flamed razor, stained for 2–5 minutes with cotton blue in lactophenol, and then washed in tap water for 15 minutes. Since the stain is specific for protoplasm, the bacterial cells were stained intensely blue, while the surrounding agar was scarcely tinted (Fig. 1).

[1] The author is indebted to W. H. Burkholder, G. C. Kent, E. E. Clayton, E. E. Wilson, J. C. Dunegan, G. L. Peltier, R. H. Bamberg, and P. E. Tilford for some of these cultures.



(*Photographs by J. A. Carlile*)

Fig. 1. Development of colonies from isolated, single cells of *Phytomonas stewarti* in poured plates. The agar from a thickly populated plate was sliced and then incubated under aseptic conditions. A, B and C. Representative fields in agar slices stained after they had been incubated for 0, 3 and $4\frac{3}{4}$ hours, respectively. Over 99 per cent of the loci occupied by bacteria immediately after the agar hardened had isolated single cells as illustrated in A, and most of these cells produced colonies as shown in B and C and Fig. 2, A and B. ×1409.

Distribution of bacteria in the agar was determined by microscopic examination of slices stained immediately after the agar hardened. The loci occupied by bacteria were classified as containing 1 isolated cell, 2 cells, or more than 2 cells. Pairs of cells attached end-to-end are listed in the tables as 2 cells, but, in the computations, they are considered as single cells, because they were obviously derived from a single cell. Pairs of cells side-by-side, end-to-side, or end-to-end with adjacent ends not in straight alignment were considered as 2 cells of different origin. Many of these pairs, however, were probably progeny of the same cell. The percentage of loci occupied by single cells was calculated and is referred to in the tables as the percentage of isolated cells. Growth of the bacteria in agar was determined by microscopic examination of agar slices stained after different periods of incubation in sterile van Tieghem cells.

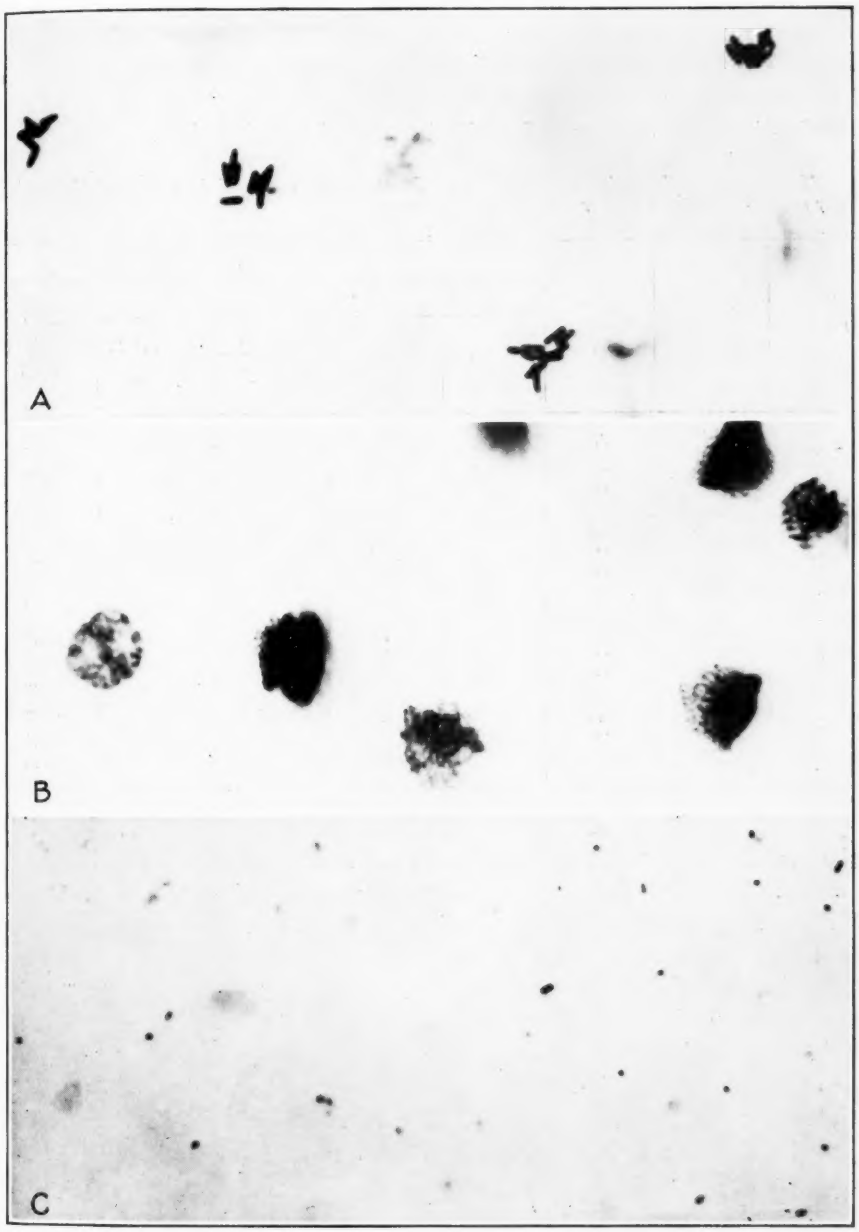
EXPERIMENTAL RESULTS

Dispersion and Growth of Phytomonas stewarti in Agar. The distribution and multiplication of cells of a highly virulent strain (B–1311) of *P. stewarti* in agar were studied. Duplicate broth subcultures were dispersed into nutrient agar containing either 0.5 or 1.5 per cent dextrose. Microscopic examination showed that the bacteria were uniformly distributed in all 4 preparations. An average of 99.2 per cent of the loci occupied had single cells (Table 1) and the remainder had pairs of cells. There was no evidence of agglutination (Figs. 1, A and 2, C).

The isolated cells grew very well in agar, only 6 to 22 per cent of them failing to multiply within 12 hours (Table 1). The bacteria grew better in the 1.5 per cent dextrose agar than in the poorer medium. They multiplied more rapidly and a larger proportion of them produced colonies in this rich medium than in the poorer one.

The development of colonies from isolated cells in unstained agar preparations (0.5 per cent dextrose) was observed under oil immersion. The cells did not change perceptibly during the first hour, they began to enlarge during the second hour, and about half of them divided by the end of the third hour. The sister cells elongated immediately and subsequent divisions occurred about every $1\frac{1}{2}$ hours. As the sister cells elongated, their ends slipped by each other (Fig. 1, B, a) in such a way that many of them rested side-by-side (Fig. 1, C, b) at the time of the second division. Occasionally short chains developed as the rapidly growing cells (Fig. 1, C, c) failed to complete the first division before the second one was initiated. The individual cells varied widely in their ability to start multiplying after the agar hardened (Fig. 1, B and C). The majority divided within 3 to 5 hours, but some did not divide until after about 12 hours of incubation. Some cells divided but the sister cells produced remained inactive. Most of the isolated cells, however, produced small colonies of 160 to 400 cells within 16 to 22 hours after they were placed in agar (Fig. 2, B).

The progressive changes that occur during the development of colonies



(*Photographs by J. A. Carlile*)

Fig. 2. Development of colonies from isolated, single cells of *Phytomonas stewarti* in poured plates. A and B. Representative fields in agar slices from the same preparation as in Fig. 1 that had been stained after incubation for 6 and 22 hours, respectively. C. Isolated, single cells in another preparation stained immediately after the agar hardened. A, × 1409. B and C, × 729.

may be illustrated by the data presented in table 1. In subculture B on 1.5 per cent dextrose agar, about 50 per cent of the cells divided within $2\frac{1}{4}$ hours, but most of them were end-to-end and few had completed the second division. Two hours later, 86 per cent of the cells had divided and most of them were side-by-side. Many cells had divided a second or third time.

TABLE 1.—*Dispersion and growth of Phytomonas stewarti in sections of nutrient agar containing different percentages of dextrose*

Subculture no.	Incubation period minutes	Percentage dextrose	Distribution of bacteria in agar				
			No. of loci with				Percentage isolated cells
			1 cell	2 cells		More than 2 cells	
				End-to-end	Side-by-side		
A	0	1.5	1257	41	10	0	99.2
	210	"	202	.....	434	2	31.7
	330	"	117	.....	104	467	17.0
	690	"	120	.....	22	905	11.4
B	0	1.5	585	18	5	0	99.2
	135	"	460	266	175	7	50.7
	255	"	127	183	451	138	14.1
	630	"	64	.....	180	735	6.5
A	0	0.5	869	71	8	0	99.1
	420	"	267	.....	290	0	47.9
	720	"	116	.....	256	162	21.7
B	0	0.5	1031	20	7	0	99.3
	180	"	341	.....	224	0	60.4
	740	"	163	.....	299	263	22.5

Other subcultures of B–1311 were suspended in melted agar, as described above, and part of the agar was poured from the test tubes into Petri dishes to harden. Examinations of 7 different preparations showed an average of $99.11 \pm .13$ per cent of the loci occupied by single cells. There was no significant difference between the preparations allowed to harden in test tubes and those in dishes. However, when suspensions were prepared by pouring melted agar on bacteria in Petri dishes, only 98.1 and 98.6 per cent of the loci were occupied by single cells.

Studies were next undertaken to determine the distribution of bacteria in plates prepared by other methods. The effect of different nutrients in the agar, the viscosity of the agar, and the method of mixing the agar and bacteria were studied.

Poured plates containing a high percentage of single cells were prepared by several different techniques (Table 2), but the routine method described above proved to be the most reliable (No. 8). Suspensions mixed in Petri dishes (No. 3) contained almost 99 per cent isolated cells when agar of proper consistency was used. However, plates prepared with agar rendered viscous by over-cooling (No. 5) or the addition of too much agar (No. 4) had only 95 to 97 per cent of the loci occupied by isolated cells. The nutrients in the

TABLE 2.—*Effect of differences in experimental procedure upon the distribution of bacterial cells (of Phytomonas stewarti) in agar*

Experiment no.	Experimental procedure							Distribution of bacteria in agar				
	History of culture			Mixed with agar				No. of loci with				
				Composition					2 cells			
	Strain no.	Grown on	Diluted in broth	Nutrient	Percentage agar	Temp. °C.	In dish	In test tube	1 cell	End-to-end	Side-by-side	More than 2 cells	Percentage isolated cells
Nutrient													
1	B–1311	Broth	+	None	1.5	42°	+	–	455	26	5	1	98.8
2	B–1311	Broth	+	Synth.	1.5	42°	+	–	449	21	6	1	98.5
3	B–1311	Broth	+	Nut. dex.	1.5	42°	+	–	1257	41	14	0	98.9
Agar viscosity													
4	B–1311	Broth	+	Nut. dex.	3.0	43°	+	–	774	62	47	0	94.7
5	B–1311	Broth	+	Nut. dex.	1.2	38°	+	–	720	32	23	2	96.8
Cultures used													
6	B–1311	Agar	–	Nut. dex.	1.5	42°	–	+	438	53	343	94	52.9
7	B–1311	Agar	+	Nut. dex.	1.5	42°	–	+	795	29	38	5	95.0
8	B–1311	Broth	+	Nut. dex.	1.5	42°	–	+	502	15	3	2	99.0
9	B–11–163	Broth	+	Nut. dex.	1.5	42°	–	+	984	33	8	0	99.2
10	A–17	Broth	+	Nut. dex.	1.5	42°	–	+	1276	36	13	2	98.9

agar apparently did not affect the distribution of the bacteria, since similar results were secured with bacteria suspended in plain water agar (No. 1), synthetic agar (No. 2) and nutrient-dextrose agar (No. 3). The three strains of bacteria, B–1311 (No. 8), B–11–163 (No. 9), and A–17 (No. 10), were equally well distributed in poured plates. The only method tested that could not be recommended (No. 6) consisted of scraping the surface growth from an agar slant and suspending the bacteria directly in melted agar without previously diluting them in broth. Under these conditions only 53 per cent of the loci occupied had single cells, and many clumps of cells existed in the hardened agar. However, when bacteria from agar slants were diluted in broth before they were placed in melted agar (No. 7), practically all of the groups disintegrated and at least 95 per cent of the loci occupied were seeded with single cells.

Single-cell Origin of Colonies of Phytomonas stewarti in Dilution Plates. It has been observed for other bacteria that many cells usually are sown for every colony that develops on dilution plates. This observation has been explained by assuming either that some cells failed to produce colonies (18) or that clumping of cells occurred (8). Since neither hypothesis has been supported by direct observation of the cells in agar, studies were undertaken to determine the difference between cell and colony counts for *P. stewarti* and to see if such differences were correlated with the failure of some cells to produce macroscopic colonies. A 16-hour-old broth culture of B–1311 was diluted serially and 1 cc. of each dilution was dispensed in each of 5 Petri dishes. The suspensions were mixed with melted agar at 42 to 45° C. and then incubated for 3 days at 26° C. Plates containing less than 1500 colonies were counted without the aid of a microscope, while the more thickly populated ones were observed microscopically. The number of cells present in the culture at the time the plates were poured was determined from 5 smears of .01 cc. each from both the original culture and its 10^{-1} dilution.

The data presented in table 3 show that 1 cc. of the original culture contained about 286,500,000 cells but produced only 119,400,000 colonies (calculated from the average of the 10^{-6} dilution). In other words, about 42

TABLE 3.—*The number of cells in smears from a broth culture of Phytomonas stewarti compared to the number of colonies that developed on dilution plates seeded with the same culture*

Dilution of culture	Units counted	No. of cells or colonies obtained from each cc.					
		1	2	3	4	5	Average
None	Cells ($\times 10^3$)	232,343	369,118	337,255	228,361	265,425	286,500
10^{-1}	Cells ($\times 10^3$)	24,510	27,633	31,421	26,581	29,881	28,005
10^{-2}							
10^{-3}	Colonies	103,356	101,635	96,130	93,020	98,421	98,512
10^{-4}	Colonies	12,632	9,613	9,751	11,656	10,751	10,881
10^{-5}	Colonies	1,208	1,184	1,256	1,160	1,080	1,178
10^{-6}	Colonies	121	112	118	122	124	119
10^{-7}	Colonies	13	13	11	16	9	12
10^{-8}	Colonies	2	1	1	1	0	1

colonies developed for each 100 cells sown. Similar results were secured with a 14-hour-old culture, but a 48-hour-old culture gave only 28 colonies for each 100 cells.

After the poured plates had been incubated for 80 hours, agar that had been mixed with the 10^{-2} dilution was sliced, stained, and examined. The loci occupied by bacteria were classified according to whether they contained more or less than 16 cells, care being exercised not to count the cells that had been spread along the cut surface of the agar. Between 38 and 55 per cent of the loci occupied by bacteria in different plates contained less than 16 cells. Apparently the difference between cell and colony counts was due, for the most part, to the failure of some isolated cells, particularly those deeply embedded in agar, to develop macroscopically visible colonies.

Separation of Mixed Strains of Phytomonas stewarti on Poured Plates. The dilution-plate method should be a reliable technique for separating mixed

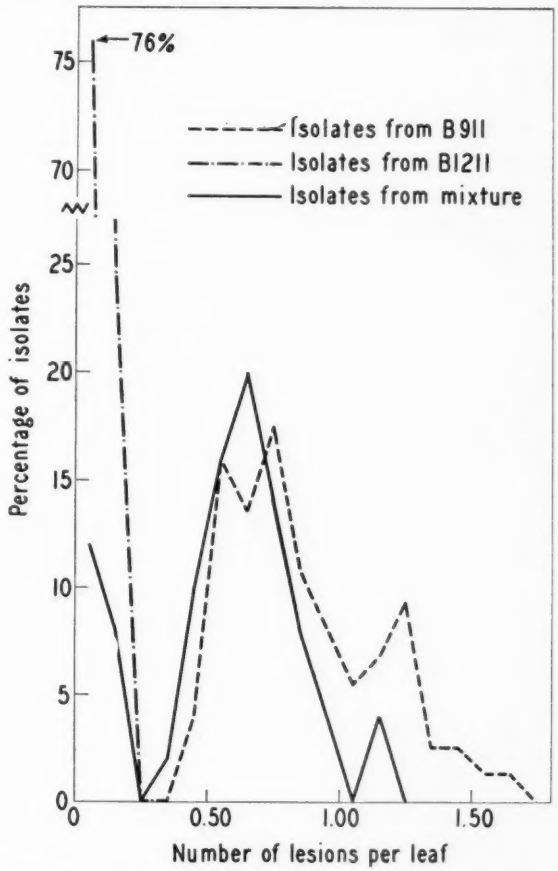


Fig. 3. Distribution of single-colony isolates from a virulent culture (B–911), a slightly virulent culture (B–1211), and a mixture of these 2 cultures. The virulence of the isolates was expressed by an infection index based upon the average number of necrotic lesions produced per leaf, and the isolates were then grouped into classes differing by 0.10 lesion. The parent types were recovered from the mixture by the dilution plate method. Seventy-four isolates of B–911, 62 of B–1211, and 45 of the mixed culture were tested.

cultures of *P. stewarti*, since almost all colonies are of single-cell origin. In order to test its reliability for separating pure lines of the bacterium, the following experiments were made. A virulent culture (B–911) and a slightly virulent one (B–1211) were seeded in separate tubes of broth, incubated for 18 hours, and then samples from the 2 were mixed in a 3rd tube. Within an hour all 3 cultures were shaken, diluted, and suspended in melted agar for dilution plates. Single-colony isolates obtained from these plates were tested for virulence. Sets of 15 to 20 sweet-corn seedlings of the variety Golden Bantam were inoculated according to the method previously described (10). The various isolates were then classified on the basis of pathogenicity, as indicated in figure 3. The data show that, although variants were present in both cultures at the time they were mixed, the isolates from the 2 fell into distinctly different classes. The isolates recovered from the mixed culture fell into either one parent class or the other and showed no tendency to form an intermediate group.

A similar experiment was made with an avirulent (B–11–163) and a highly virulent (B–1311) culture. Forty-four single-colony isolates from each parent culture and 88 from the mixture of the 2 were tested for virulence on 6-day-old sweet-corn seedlings. About 18 plants were inoculated with each isolate and the percentage of invaded leaves was computed from records taken 15 days after inoculation. The plants were then harvested and dried to constant weight at 102° C. The data obtained (Table 4) show that none

TABLE 4.—*Classification of the single-colony isolates from an avirulent culture (B–11–163), a virulent culture (B–1311), and a mixture of the two cultures according to the percentage of leaves invaded in 6-day-old sweet-corn seedlings*

Leaves invaded (Per cent)	Isolates from B–11–163		Isolates from B–1311		Isolates from mixture	
	Number	Dry weight	Number	Dry weight	Number	Dry weight
0–09	44	.299[a]	0	.....	52	.347
10–19	0	.....	0	.....	0	.....
20–29	0	.....	0	.....	0	.....
30–39	0	.....	7	.347	2	.265
40–49	0	.....	3	.297	7	.246
50–59	0	.....	6	.257	6	.237
60–69	0	.....	17	.226	10	.206
70–79	0	.....	9	.227	8	.159
80–89	0	.....	1	.230	2	.170
90–99	0	.....	1	.140	1	.100
Uninoculated controls	3	.310	3	.375	3	.327

[a] Average dry weight per plant in grams.

of the isolates from B–11–163 invaded the plants or caused a severe reduction in dry weight. The isolates from B–1311, however, invaded from 33 to 97 per cent of the leaves and caused a pronounced reduction in dry weight. Although the isolates from B–1311 differed in virulence, the least virulent one was distinctly more virulent than any of those obtained from culture B–11–163.

The mixed culture yielded 52 isolates of the B–11–163 type. None of these caused lesions or severely reduced the growth of the plants. The remaining 36 isolates were similar to those obtained from culture B–1311. Nine of them were of the weakly virulent type that invaded only 30 to 50 per cent of the leaves, while the remaining 27 isolates were of the more virulent type. These data bring additional evidence that colonies on poured plates develop from isolated, single cells.

Dispersion and Growth of Other Species in Agar. Observations similar to those described above were made on other phytopathogenic bacteria, since it was thought that the results obtained with *Phytomonas stewarti* might not apply to other species. The species listed in table 5 were tested for dispersion and growth in agar. The first 9 of these species gave results very similar to those obtained with *P. stewarti.* Over 97 per cent of the loci occupied had single cells. Some of the cultures that grew very rapidly, such as *Erwinia carotovora, P. pisi, P. angulata,* and *P. savastanoi,* had a large number of cells in late stages of division (end-to-end) at the time the agar hardened. *P. translucens* var. *undulosa* was the only one of these species in which many of the isolated cells failed to grow.

The 3 strains of *Phytomonas tumefaciens* and the cultures of *P. insidiosa, P. michiganensis,* and *P. juglandis* had 89 to 96 per cent isolated cells. These cultures also formed a few clumps of from 3 to 80 cells each. The isolated single cells from these 4 species grew readily and produced colonies within 20 hours. Many of the cells of *P. fascians,* the causal agent of sweet-pea fasciation, were clumped together in the agar and only 51 per cent of the loci occupied had single cells. This bacterium may have given results different from those secured with other species because of its different growth habits. On agar it at first produces a thin, spreading, filamentous type of growth that later becomes wrinkled and crustaceous. In broth it produces a thin, granular pellicle and many of the cells collect at the bottom of the tube in clumps; so that there is very slight, if any, clouding of the medium.

Broth suspensions of the 5 cultures that had less than 97 per cent isolated cells were examined in hanging drops. Clumps and pairs of cells were found to exist before the bacteria were placed in agar. Actual counts indicated that there were more groups of cells in the broth than in the agar mixed with the same suspensions. This difference is not considered significant, however, since it was impossible to secure reliable counts of the unstained cells while they were moving about in hanging drops. This evidence shows that agar does not cause agglutination of any of the bacteria tested.

DISCUSSION

Koch (9) stated, after he had described the poured-plate method, that he was unable to determine whether or not colonies developed from isolated cells because the thick layer of gelatine prevented microscopic examination. This handicap has been overcome by the simple methods used in these studies. Direct observations have confirmed Koch's assumption that most colonies

TABLE 5.—*Dispersion and growth of different species of phytopathogenetic bacteria in nutrient-dextrose agar*

Bacteria tested	Before incubation					After 20 hours in agar			
	No. loci with				Percentage isolated cells	No. loci with			Percentage isolated cells
	1 cell	2 cells		More than 2 cells		1 cell	2 cells	More than 2 cells	
		End-to-end	Side-by-side						
E. carotovora	803	37	17	4	97.6	2	2	742	0.3
P. campestris	1042	26	12	6	98.3	78	23	699	9.7
P. phaseoli	1323	31	21	10	97.8	88	150	418	13.4
P. angulata	853	57	16	1	98.2	12	4	751	1.6
P. savastanoi	732	109	8	5	98.5	13	8	439	2.8
P. pisi	536	176	9	1	98.6	6	1	589	1.0
P. tabaca	769	21	2	1	99.6	38	17	567	6.1
P. pruni	856	35	2	2	99.6	38	3	531	6.6
P. translucens var. *undulosa*	1013	32	8	4	98.9	644	130	217	65.0
P. tumefaciens	880	47	27	18	95.4	11	2	811	1.3
P. tumefaciens	1043	64	45	9	95.3	11	3	1055	1.0
P. tumefaciens	938	60	38	6	95.8	8	1	938	0.8
P. juglandis	602	26	30	26	91.8	31	27	705	4.1
P. insidiosa	774	63	76	25	89.3	24	77	882	2.4
P. michiganensis	937	69	59	26	92.2	27	10	956	2.7
P. fascians	252	123	0	354	51.4	50	15	383	11.2

develop from isolated, single cells. Since the effectiveness of the poured plate has been well established by 55 years of universal usage, further evidence in support of its reliability as a method for the isolation of cultures derived from single cells hardly seems necessary. The data presented in this paper are offered in the hope that they will be of use to those who may wish to properly evaluate criticisms of the method. Burri's (3) argument that the single-cell origin of any particular colony is hypothetical must be accepted, because there are a few colonies that develop from more than 1 cell. None of the data obtained, however, support those who claim that colonies rarely, if ever, develop from isolated cells.

Although several phytopathogenic bacteria have been studied, other species may give different results. Cultures that produce either heavy pellicles, strings of slime, or sediment in broth may well be suspected of containing clumps of cells. Such clumps might be avoided by using young cultures for inoculum and by using special devices (6) to shake them apart; but to be certain that they are not present, cultures should be examined in hanging drops before being dispersed in agar. According to the observations reported above, the unattached cells in broth remain separated when placed in melted agar. Although other species might be agglutinated by melted agar, no data in support of such an assumption have been found in the literature. Apparently this concept arose from a misunderstanding of Smith's (14) observation that the water of syneresis from solidified agar contained a diffusible material that caused some bacteria to agglutinate. He did not discuss bacteria in melted agar. Thermo-agglutination of bacteria has been reported (5, 2), but this phenomenon does not apply to the poured plate in which the bacteria are not exposed to a lethal temperature.

The single-cell origin of most cultures may be guaranteed by making several serial dilutions and single-colony isolations, as was done with *Phytomonas stewarti* (10). In each dilution, over 99 per cent of the loci occupied would have single cells and about 80 per cent of these cells would grow. Even if all paired cells multiplied, there would be less than 1 colony of multicellular origin for 80 of single-cell origin. After 5 successive dilutions, there would be less than 1 chance in about 3.5 billion that the culture had not been reduced to a single cell in at least one dilution. It is questionable whether even a trained observer can be any more accurate than this in determining that one, and only one, cell is floating in a hanging drop used for obtaining single-cell cultures under microscopic control.

The conclusions reached in this paper need not, necessarily, discourage the use of other pure-culture methods. There is apparently a need for both the poured-plate and other methods employing microscopic control, since each has its advantages and disadvantages. As a general rule, the poured plate will be preferred because of its simplicity. If a culture does not produce colonies of single-cell origin, it would be desirable to employ methods such as Tilford (16) used with *Phytomonas fascians*. Riker *et al.* (12, 19) obtained an occasional mixed culture of *P. tumefaciens* and *P. rhizogenes*

R., B., W., K. and S. on poured plates. It is possible that *P. rhizogenes* produced clumps of cells that involved *P. tumefaciens*, or that the strain of *P. tumefaciens* used differed from the 3 employed in these tests. Cultures of this bacterium that contain many of the star-shape bodies described by Stapp (15) might yield numerous colonies of multicellular origin.

The methods that employ microscopic control, on the other hand, also have disadvantages that have been summarized by Hort (7) and Topley, Barnard, and Wilson (17). The reliability of the method depends upon the ability of the operator to see every cell in the preparation. Since the cells are not stained, it is obvious that these methods are unsuitable for obtaining cultures of small bacteria, such as those described by Nelson (11). There would be also considerable doubt about the reliability of the method for cultures that contain involution forms. Insofar as the writer is aware, no data have been published on the reliability of the hanging-drop methods.

SUMMARY

Studies on the distribution and growth of bacteria in agar suspensions have shown that the poured-plate technique is a satisfactory method of obtaining single-cell cultures.

In plates sown with *Phytomonas stewarti*, over 99 per cent of the loci occupied by bacteria had only one cell. Eighty to 94 per cent of these isolated cells multiplied, depending on the nutrient content of the agar. The most reliable of the several methods tested for preparing poured plates with this bacterium was to suspend a young broth culture in melted agar (1.5 per cent agar at about 42° C.) and then pour the agar into a plate. Satisfactory plates were prepared, however, by several other methods in which uniform suspensions of the bacteria were placed in agar of the proper viscosity.

In plates sown with *Erwinia carotovora*, *Phytomonas campestris*, *P. phaseoli*, *P. angulata*, *P. savastanoi*, *P. pisi*, *P. tabaca*, *P. pruni*, or *P. translucens* var. *undulosa*, over 97 per cent of the loci occupied by bacteria had single cells. The isolated cells grew very readily in all species except *P. translucens* var. *undulosa*. In plates sown with *P. tumefaciens* (3 strains), *P. juglandis*, *P. insidiosa*, and *P. michiganensis*, slightly lower percentages (89 to 97) of loci were occupied by single cells. Practically all of the isolated cells multiplied. *P. fascians* was the only species studied that produced many clumps of cells in agar. Examination of broth suspensions of the 5 species that had less than 97 per cent of the loci occupied by single cells showed that the pairs and clumps of cells existed before the bacteria were placed in the melted agar.

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THE TUMOR DISEASE OF OAK AND HICKORY TREES

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INTRODUCTION

The writer's acquaintance with tumors on oak trees dates back to early 1912, when a surgeon connected with the School of Medicine of Boston University submitted a culture of a bacterial organism he had isolated from a tumor on red oak. This culture he wished to have compared with *Bacterium tumefaciens*. A report of his study of the oak disease, made to the Medical Society of Boston in 1911, stated that he believed the oak tumors to be of bacterial origin and that the organism was *Bact. tumefaciens* (6),

the cause of tumors on many varieties of plants. Accompanying his culture were some photographs of large galls on oak trees.

A comparison of the surgeon's organism with *Bacterium tumefaciens* was made, as he requested, and it showed that the two organisms were unrelated. In the spring of 1912, over 40 inoculations were made with his oak

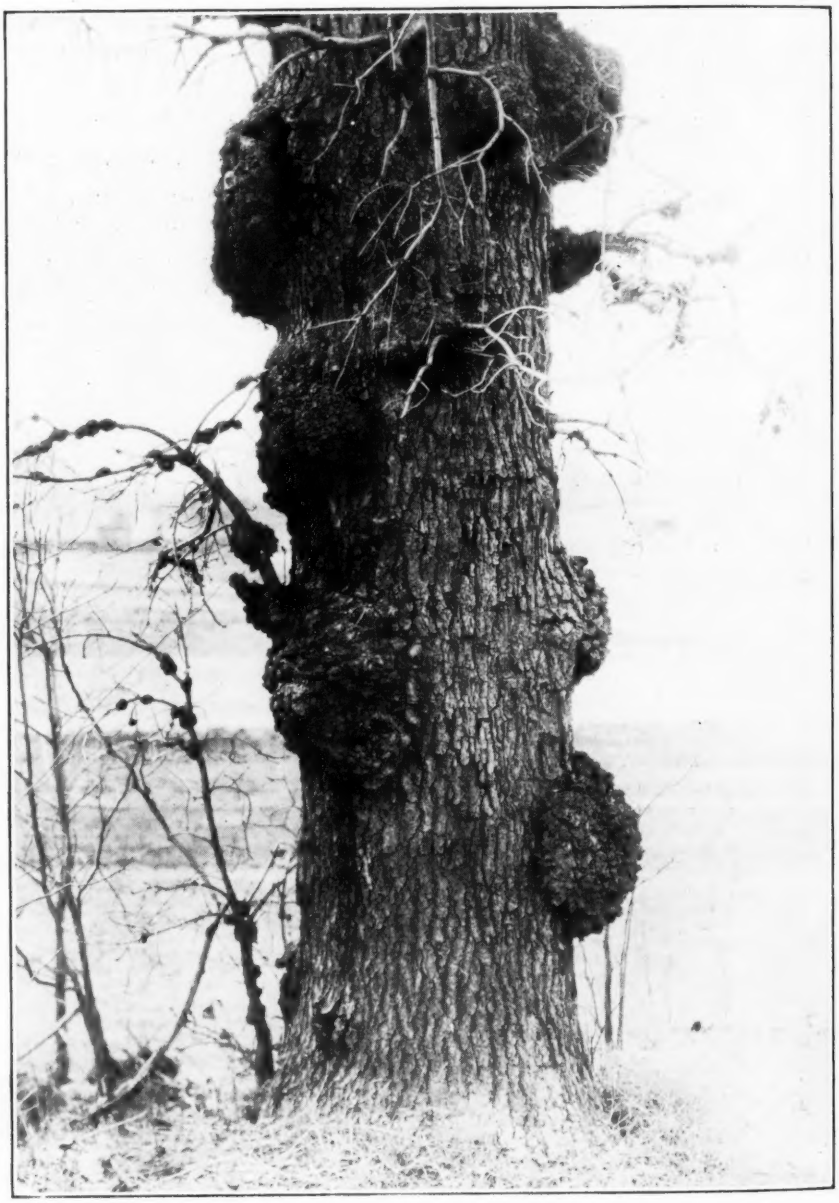

FIG. 1. Galls on black oak, from which a Phomopsis was isolated, in Prince Georges County, Md.

isolation into 5 varieties of oaks, some growing out-of-doors, others in the greenhouse. No infections followed. Other plants susceptbile to *Bact. tumefaciens* were inoculated with the oak organism from Boston, with negative results. Assuming that the disease might really be due to some strain of *Bact. tumefaciens* and that the surgeon had not succeeded in isolating the infectious organism, oak trees on the grounds of the Department of Agriculture and others growing in the greenhouse were inoculated with several strains of *Bact. tumefaciens.* No galls developed on the 30 young oak twigs that were inoculated; and it was concluded that the cause of the disease must be looked for elsewhere. Two years later, when oak galls were received from several localities, they were referred to the Bureau of Entomology and Plant Quarantine. The report of that Bureau stated that the galls were not of insect origin.

As the interest in oak tumors developed, outgrowths of the same type were found on hickory (*Hicoria*) trees. It should be stated here that some time before any work was done on hickory galls the writer isolated *Bacterium tumefaciens* from a gall on the pecan, *Hicoria pecan.* Typical crown galls were reproduced by inoculations with the pecan isolation into a wide variety of plants. The hickory tree was not included, although other strains of *Bact. tumefaciens* have since been inoculated into hickory, without success. The crown gall on pecan is not at all common, and the specimen received was on a tree grown in an old peach orchard. It is probable that the orchard was well seeded with *Bact. tumefaciens.*

DESCRIPTION OF THE GALLS

The oak tumors and those of the hickory resemble crown gall in their external appearance (Fig. 1, Fig. 2, A and B). They are usually dark colored and nodular and range from 1 to 100 on a tree (Fig. 2, C). With the exception of the youngest proliferating ones, the galls are of hard wood covered with rough bark. The size varies from less than $\frac{1}{2}$ in. (Fig. 3, A) to tumors over a foot in diameter (Fig. 3, B).

EXPERIMENTAL STUDIES

Attempts to Isolate Bacterium tumefaciens

Although the galls have generally been considered by pathologists and entomologists to be those of crown gall, the validity of the assumption had not been established; so research was undertaken to ascertain if a pathogen was responsible and, if so, whether the pathogen was of bacterial or fungal origin. Isolations were made from oak and hickory galls received from 7 States, including 28 lots of material, 12 of which came from Virginia and Maryland. These isolations spread over a period of years but not consecutive ones. No crown-gall colony appeared on the hundreds of plates made from young galls, nor did other bacterial colonies appear in sufficient numbers to indicate that the galls were of bacterial origin.

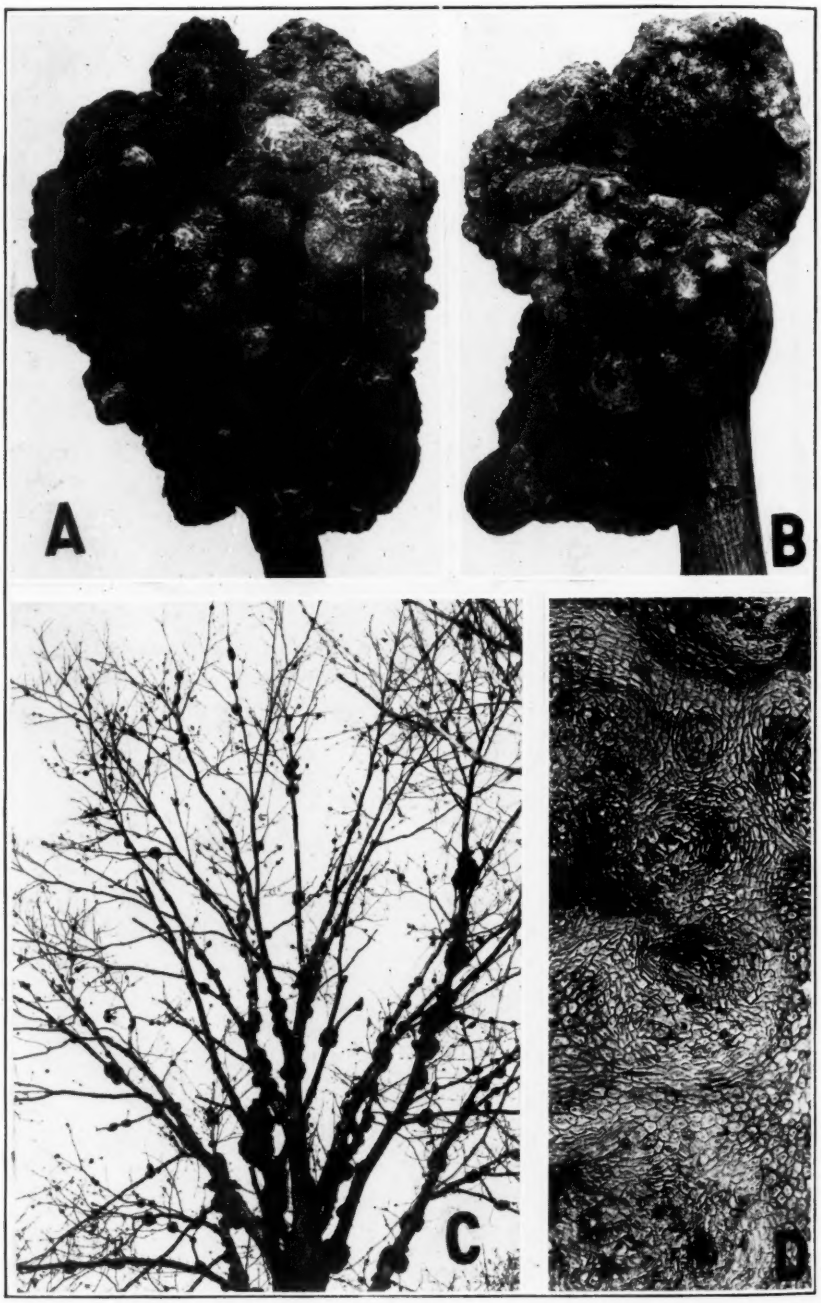

FIG. 2. A. Chestnut oak gall from Arlington County, Va. B. Galls on hickory near galled chestnut oak, Arlington County, Va. C. Red oak galls from Montgomery County, Md. Large galls occurred on the trunk. D. Portion of a stained section of a young chestnut oak gall. A and B. Approximately natural size. C. Much reduced. D. About ×110.

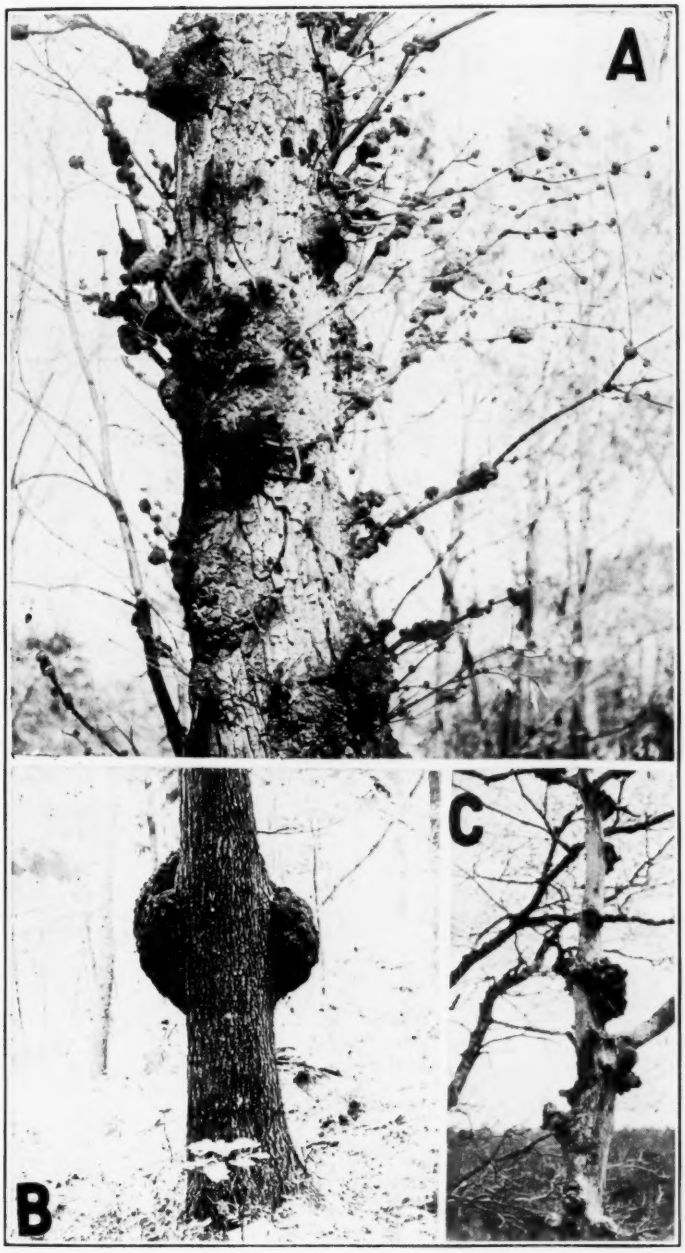

FIG. 3. A. Chestnut oak galls in Frederick County, Va. B. Large tumor on black jack oak, Arlington County, Va. Circumference of gall 4 ft., 11 inches. C. Red oak galls on dead trees in Massachusetts.

A Fungus Isolated from the Galls

While plating for *Bacterium tumefaciens*, fungus colonies frequently appeared and, because similar colonies grew on the plates poured from different lots of material, attention was directed to them for further study. This fungus produced pycnidia bearing tiny Phoma-like spores. In the years through which this investigation extended, intermittent though it was, this type of spore was isolated from all the galls that were studied immediately after they were taken from the trees.

Sections of galls were studied and mycelium was found in young growing galls, usually between the cells. The mature gall tissue appeared to be made up largely of xylem elements and groups of xylem cells. Part of a section of a young chestnut oak gall showing marked disarrangement of cells is shown in figure 2, D.

The Fungus Identified as a Phomopsis

Up to the present time, in the study of living oak and hickory galls, no fruiting bodies have been seen on the surface nor have the galls produced either pycnidia or perithecia when placed in a moist chamber. Galls kept out-of-doors overwinter, subjected occasionally to freezing temperatures and temperatures as low as 12° F. for short periods of time, did not produce pycnidia when brought indoors and placed in moist chambers. However, isolations made directly from the gall tissue exposed to the elements overwinter, produced pycnidia bearing *Phomopsis* spores of types *a* and *b*. Also the Phoma-like cultures kept out-of-doors overwinter and at the same temperatures as the galls mentioned above, produced both *a* and *b* types of *Phomopsis* spores when they were later placed at room temperatures. It is believed, however, that there are conditions in which pycnidia are produced on the surface of oak and hickory galls as they are on those of privet and viburnum, although the writer has not seen them.

For a number of years before the *Phomopsis* spores were obtained in culture, the Phoma-like pycnospores from various isolations were inoculated into oak and hickory stems, but nothing more than slight swellings resulted. Over 500 inoculations were made with the Phoma-like spores. With the development of the *Phomopsis* spores and better inoculation technique, the work progressed favorably.

Inoculations with Phomopsis Cultures

The *Phomopsis* spores that developed from the cultures containing Phoma-like spores proved to be infectious and produced galls on oak, hickory, *Viburnum opulus*, *Ligustrum vulgare*, *Jasminum nudiflorum* (Fig. 4, C–J) and the cultivated blueberry, *Vaccinium corymbosum*. The last 4 species are subject to a gall disease that has been reported as caused by a *Phomopsis* (1, 2, 3). Cultures of the *Phomopsis* of both oak and hickory produced galls quite readily on viburnum, privet, and jasmine; much more

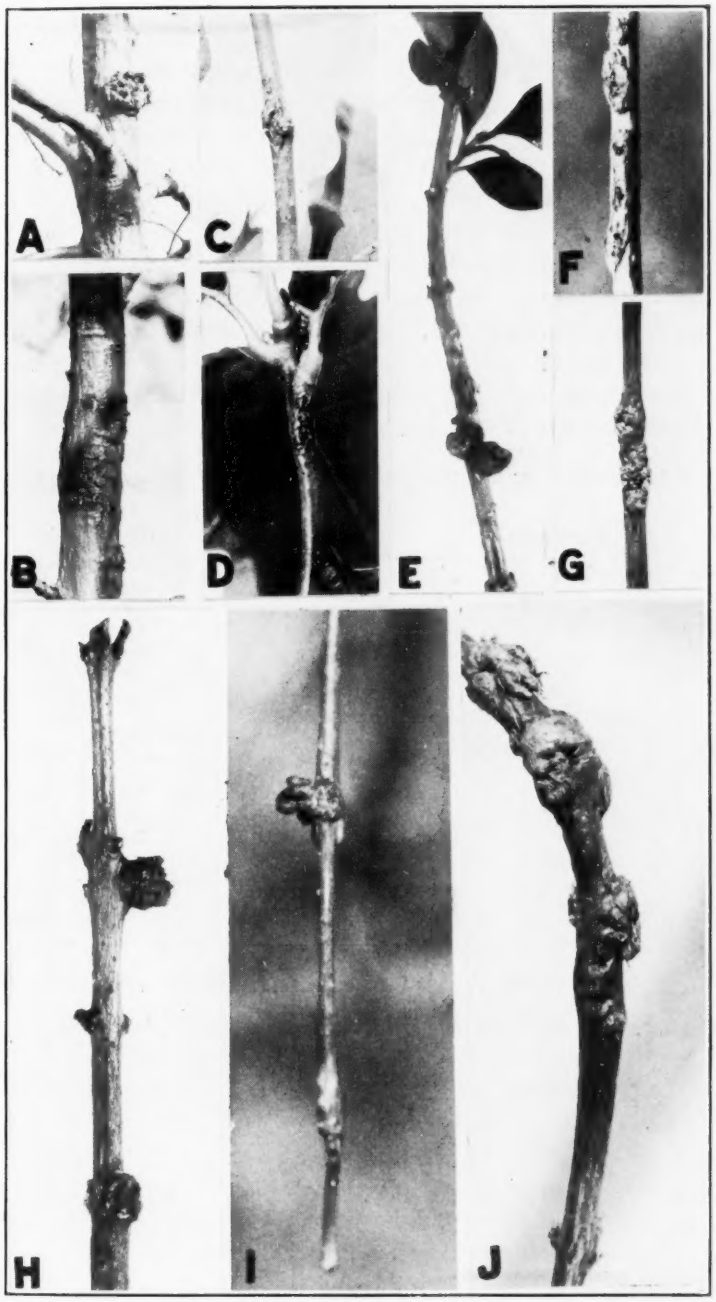

FIG. 4. A and B. Beginning stages of gall on black oak trees in Prince Georges County, Maryland. Phomopsis isolated from this material. C and D. Seedling oaks in greenhouse inoculated 7–20–37 with *Phomopsis* isolated from black oak. Photographed 9–24–37. E. Privet stem inoculated with oak *Phomopsis* 5–16–36. Photographed 8–24–36. F. Stem of *Viburnum opulus* inoculated with oak *Phomopsis* 5–20–36. Photographed 11–5–36. G. *Jasmine nudiflorum* inoculated with oak *Phomopsis* 4–29–37. Photographed 9–26–37. H. Privet stem inoculated 5–22–36 with *Phomopsis* isolated from hickory gall. Photographed 8–24–36. I. *Viburnum opulus* stem inoculated with hickory gall *Phomopsis* 5–22–36. Photographed 11–5–36. J. *Jasmine nudiflorum* inoculated with hickory gall *Phomopsis* 4–12–37. Photographed 9–24–37. All slightly reduced. *Phomopsis* was reisolated from all except A and B.

readily on viburnum and privet than on either oak or hickory. It is possible that one of these shrubs may be the original host of the oak and hickory gall-producing organism. The oak *Phomopsis*, likewise, produced galls on the cultivated blueberry. The hickory strain was not tested on blueberry.

The hickory *Phomopsis* used as inoculum produced small galls on hickory and oak stems. This strain seemed to be less infectious than the one isolated from the oak. From these galls, reisolations were made and the Phoma-like type of spores was again obtained in culture. Similar reisolations from the viburnum, privet, and jasmine galls produced with both oak and hickory *Phomopsis* cultures were, likewise, made successfully. Table 1 includes inoculation records of 1936 and 1937, in which both the oak and hickory *Phomopsis* were used.

TABLE 1.—*Inoculations into various hosts with oak and hickory gall Phomopsis*

Oak Phomopsis			Hickory Phomopsis		
No. of inoculations into		No. of galls	No. of inoculations into		No. of galls
Black oak	52	16	Hickory	45	4
Chestnut oak	47	19	Chestnut oak	16	8
White oak	21	4	White oak	17	4
Red oak	4	0	Black oak	13	2
Pin oak	19	0	Pin oak	0	0
Other hosts inoculated			Other hosts inoculated		
Hickory	17	2			
Viburnum	16	14	Viburnum	12	4
Jasmine	11	7	Jasmine	6	4
Privet	23	11	Privet	13	7
Blueberry	9	4			
Total	219	77 or 35% takes	Total	124	33 or 27% takes

NOTES ON MAPLE AND ELM GALLS

Galls on maple and elm trees have been studied also. No crown-gall colonies were isolated, but the fungus producing Phoma-like pycnospores was obtained from both hosts. Inoculations with the maple isolation were made into silver, red, and ash-leaved maples in Washington, D. C., by the writer, and in Ann Arbor, Michigan, by Winifred E. B. Chase. In all, 72 inoculations were made in the spring of 1921. Some swellings resulted from the Washington inoculations but no galls. Dr. Chase's results also were negative. Some of the inoculated ash-leaved maple stems were received from her in the late summer of 1921 and, although there were no outgrowths, micro-sections through the inoculated areas showed pycnidia with the tiny Phoma-like spores. These were embedded in tissue that grew around them. Isolations were made from this material and a fungus like the original one sent to Dr. Chase developed on the plates. If the inoculation technique used in 1937 had been developed at the time the work was done on the maple seedlings and trees, the results might have been not altogether negative.

In the fall of 1937, isolations were made again from elm galls and the same fungus was obtained, as in previous years. After chilling the cultures for some weeks *Phomopsis* spores developed. In the spring of 1938, inoculations with this strain will be made into elm trees and other hosts, to try to determine its pathogenicity.

Entrance of Pathogen and Spread of Infection

The parasite apparently is a weak one, for it has to enter the stem through a wound, the tissue must be young and growing well, and the organism must have moist conditions until it becomes established. Although no pycnidia have been seen on the surface of these galls, they no doubt are formed under certain conditions. Since the enlargement of the gall results mostly through the proliferation of its outer layers of tissue, the older hyphae capable of producing fruiting bodies are not close to the surface. A live gall sooner or later should produce fruiting bodies. In cultures the spores exude from the pycnidia in long tendrils if the medium is dry, and in wide spore masses if enough moisture is present. The spores are held together by a gelatinous substance and apparently are not carried far by air currents. This factor, together with the scarcity of surface pycnidia, may explain why the disease is one of limited extent.

Throughout this investigation the writer has not found any widespread development of galls in oak and hickory trees. A thicket of trees occasionally is infected but usually the slow-growing tumors are found on a few trees or an isolated one here and there in a forest, a park, or home acreage. Hickory trees in the neighborhood of badly diseased oaks have been found galled from the surface of the ground to the topmost branches. The infection works slowly, however, and it takes years to kill a tree (Fig. 3, C). According to the farmer on whose land the tree (Fig. 1) is growing, it has been nearly dead for about ten years. Its branches still continue to bear leaves. Various species of oaks have the disease, but the writer has found the tumors more frequently on the chestnut and black oaks.[1]

Beginning stages in the growth of the gall on the main trunk of black oak are shown in figure 4, A and B. They have somewhat the appearance of young cankers. These were found on a young black oak tree growing about 15 feet from the mature infected black oak shown in figure 1. There were several branches on the young tree with this beginning stage and a few with galls an inch in diameter. Isolations were made, using the stage pictured in figure 4, A, from which a fungus with Phoma-like pycnospores was readily obtained. After chilling these cultures in the refrigerator for a few weeks, *Phomopsis* spores developed. In the spring of 1938 these cultures will be used for inoculations into susceptible hosts.

The slow growth of the galls and the slow spread of the infection contribute to an easy control of the disease by simply cutting and burning the diseased parts or destroying the whole tree.

[1] In the spring of 1938 leaves developed on some branches but were dead by the middle of May.

PERFECT STAGE OF THE PHOMOPSIS NOT OBTAINED

Up to the present, the perfect stage of the fungus has not been found. Repeated attempts to produce this stage were made by placing galled stems in wire screening on the ground under oak trees and covering with oak leaves; by sinking wire baskets containing galls in snow; by leaving waterproof-covered cultures exposed to winter temperatures from one to several months; by culturing the fungus in a wide variety of artificial media; by culturing on sterile oak leaves and stems in tubes; by sterilizing oak and hickory stems in mercuric chloride 1 to 1000 for 10 minutes, washing in sterile water, inoculating with various strains of *Phomopsis* and placing them in sterile test tubes; by inoculating oak bark, both sterilized and nonsterilized; by streaking corn meal agar plates with different strains of *Phomopsis*, as oak and hickory, oak and viburnum, etc., with the hope of obtaining + and − strains with the production of perithecia; by subjecting cultures alternately to warm and then cold temperatures, and *vice versa;* and by drying cultures, then moistening them, and *vice versa*. Galls were taken from the trees at different times of the year and examined, but no perithecium was found that could be considered related to *Phomopsis*.

GALLS AND CANKERS OF OAK NOT MANIFESTATION OF THE SAME DISEASE

In the tumor disease of oak and hickory there is a distinct outgrowth. The canker diseases of oak described in literature need not be confused with it. The goitre disease of oak, pine and fir, occurring in Germany, however, may be the same or a related disease. In the latter, the outgrowths are stated to be of considerable size and either remain covered with more or less normal bark or become a broken-down canker to which wound organisms have access.

Sprengel (7, 8), who is studying goitre, makes the statement that there is no clue to the cause, but most of the evidence indicates it may be due to an hereditary predisposition of certain trees to produce tumors in response to certain environmental factors.

Harshberger (5) refers to tumors on oaks as large as a man's head or a large pumpkin, caused by the fungus *Dichaena strumosa*. A letter from Dr. Harshberger, written March, 1922, in response to an inquiry by the writer, stated that his authority for the statement in his book was J. B. Ellis of New Jersey. In *North American Pyrenomycetes*, Ellis (4) states that oak galls caused by *Dichaena strumosa* Fries, are common on limbs and trunks of living *Quercus coccinea* and *Q. nigra*, in Carolina (Ravenel), Mexico, and New Jersey (Ellis), and are very injurious, finally killing the trees. He describes the perithecia of *D. strumosa*, but ends in stating that "We have never found ascigerous specimens of this or *D. faginea*." The writer hopes some day to find perithecia of a *Diaporthe* on the oak and hickory tumors and, through culturing the ascospores, prove the *Phomopsis* of these galls is associated with it.

Some comparative studies have been made of the different gall-producing strains of *Phomopsis* as to colony and pycnidia formation and size of *a* and *b* spores, but much remains to be done. These comparisons will be reported later.

SUMMARY

The tumor disease of oak and hickory is caused by a parasite, the fungus, *Phomopsis*.

The two strains are cross inoculable. They are also pathogenic to and produce galls more readily on viburnum, privet, and jasmine.

Three types of pycnospores are produced. In isolating from a gall taken directly from a tree, Phoma-like spores are formed first. Later, after chilling, these cultures produce *Phomopsis* spores of *a* and *b* types.

Up to the present, no perfect stage of the fungus has been found in nature or in culture.

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THE RUST OF STONE FRUITS[1]

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INTRODUCTION

The rust of stone fruits, caused by *Tranzschelia pruni-spinosae* (Pers.) Diet.,[2] is of considerable economic importance in many parts of the world. It was reported from Australia (24) in 1890 and subsequently by McAlpine (21, 22, 23) and numerous later investigators. Cunningham (8) has discussed its occurrence in New Zealand, and Fikry (12) recently has studied its

[1] Cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arkansas Agricultural Experiment Station. This is abridged from a thesis presented to the Faculty of the Graduate School of the University of Arkansas in partial fulfillment of the requirements for the degree of Master of Science.

[2] This fungus is also called *Puccinia pruni-spinosae* Pers. by some investigators.

prevalence in relation to the rise and fall of the water table in the Nile delta of Egypt. Butler and Bisby (5) report its occurrence in India, and it is known to be widely distributed in Europe and North America. In addition typical specimens have been examined from China, Japan, Java, Uganda, Colombia, Venezuela, Brazil, Uruguay and the islands of the West Indies.

In the southeastern portion of the United States the disease rarely appears before midsummer and is confined entirely to the leaves. Since fruit and twig infections are unknown in this section, a premature defoliation is the only visible effect of the disease. In the western portion of the country, on the other hand, Pierce (26), Goldsworthy and Smith (15), and others have reported that it frequently appears a few weeks after the leaf buds unfold. These early spring infections are derived from overwintered lesions on the twigs and, once established on the leaves, the disease is commonly present throughout the growing season. Moreover, under certain environmental conditions, the fungus attacks the fruit and causes serious financial losses.

In Europe the disease does not appear until mid-summer, according to Brooks (4) and Ducomet (9); while in Australia and New Zealand, McAlpine (22) and Cunningham (8) have reported serious injury to the fruit, as well as premature defoliation.

In 1925 the writer began a study of the rust fungi found on various species of *Prunus* to determine why there were such marked variations in the behavior and importance of the disease. While the results furnish only a partial explanation of this variation, the demonstration of the difference, hitherto not clearly realized, between the forms of *Tranzschelia pruni-spinosae* attacking the wild and cultivated species, the resultant clarification of host relations, and the revision of the present taxonomic concept of the causal organism are all related problems whose solution will aid indirectly in explaining the variations noted in the behavior and importance of this rust.

MATERIALS AND METHODS

The studies herein reported may be divided into two parts. First, a series of inoculation experiments with urediospores were performed over a period of years to ascertain the interrelation between the forms on various cultivated species and between those attacking the cultivated and wild species. In addition, the inoculation experiments with aeciospores performed by other investigators were repeated to determine more precisely the relation between the aecial stages on various species of Ranunculaceae and the uredial and telial stages on wild and cultivated species of *Prunus*. Since these inoculation experiments involved the use of living material from various parts of the country, they were carried out in Petri dishes, with the inoculated leaves floating on sugar solutions, to eliminate the possibility of introducing infectious material into Georgia and Arkansas orchards.

In the second part a detailed examination was made of 261 specimens of *Tranzschelia pruni-spinosae* from the Division of Mycology and Disease Survey, United States Department of Agriculture, as well as 128 additional

specimens in the writer's private herbarium. The host, locality, collector, and date of collection shown on the herbarium label were recorded for all collections and a slide was prepared from each collection showing teliospores. From these data it was possible to make certain groupings of the hosts and arrive at conclusions concerning host relations that would not be evident from the casual examination of isolated specimens.

RESULTS

Inoculation Experiments with Urediospores

In 1905 Arthur (1) was unable to infect peach, *Prunus persica* S. & Z., even ". . . under seemingly the most favorable conditions . . ." with urediospores from wild cherry, *P. serotina* Ehrh. leaves. During the period from 1925 to 1927 the writer obtained similar results at Fort Valley, Georgia, and found also that the urediospores produced on peach leaves would not infect the leaves of wild cherry, *P. serotina*.

During the period 1935–1937 urediospores from various wild and cultivated hosts were used in inoculation experiments at Fayetteville, Arkansas. The results (Table 1) of these experiments confirm the previous experiments of Arthur (1) and the writer and prove that 2 forms of the rust exist. One form is able to infect only the cultivated species and the other attacks only the wild species. Since the peach was successfully inoculated in Arkansas with urediospores from a number of cultivated species from Georgia, Texas, California, and Oregon it is evident that the form attacking the cultivated species is widely distributed throughout the United States.

Inoculation Experiments with Aeciospores

Additional evidence that there are 2 distinct forms of the rust fungus on the genus *Prunus* was obtained in the inoculation experiments with aeciospores from the various alternate hosts. In 1905–06 Arthur (1, 2) successfully inoculated *Prunus serotina* and *P. pumila* L.[3] with aeciospores of *Aecidium hepaticatum* Schw., from *Hepatica acutiloba* DC. (*H. acuta* (Pursh) Britton), but failed to infect *P. americana* Marsh., *P. cerasus* L. and *P. persica*, leaves with these aeciospores. In commenting upon these results he stated,

". . . It is not possible to state what significance is to be attached to the failure to infect peach, plum, and cultivated cherry with spores that readily infect the wild cherry . . .",

and later, Jackson (17) suggested that these results indicated the presence of distinct strains of the fungus in the United States.

Clinton and McCormick (6) and the writer (Table 2) successfully inoculated wild cherry, *P. serotina*, leaves with aeciospores from *Anemone quinquefolia* L. and *A. caroliniana* Walt., respectively, but were unable to infect peach

[3] In his latest publication Arthur (3) has substituted *Prunus nana* for *P. pumila* in the report of these experiments. No authority is given for the binomial *P. nana* but it is inferred that he used *P. nana* Du Roi (= *P. virginiana* L.) in these experiments.

TABLE 1.—*Inoculation experiments with urediospores of Tranzschelia pruni-spinosae at Fayetteville, Ark., 1935–37*

Date	Inoculum		Host inoculated	Results
	Host	Source		
April 12, 1935	*Prunus serotina*	Arkansas	*Prunus serotina*	Positive, uredia produced
do.	do.	do.	*P. persica*	Negative
April 25, 1935	do.	do.	*P. serotina*	Positive, uredia produced
do.	do.	do.	*P. persica*	Negative
June 3, 1935	do.	do.	do.	Negative
do.	do.	do.	*P. serotina*	Positive, uredia produced
do.	do.	do.	*P. angustifolia* Marsh.	No results, leaves molded
June 7, 1935	do.	do.	*P. serotina*	Positive, uredia produced
do.	do.	do.	*P. persica*	Negative
do.	do.	do.	*P. communis*	Negative
August 5, 1935	*P. munsoniana* W. & H.	do.	*P. munsoniana*	Positive, uredia produced
do.	do.	do.	*P. persica*	Negative
Sept. 5, 1935	*P. persica*	Texas	*P. persica*	Positive, uredia produced
do.	do.	do.	*P. serotina*	Negative
do.	do.	do.	*P. munsoniana*	Negative
Oct. 21, 1935	do.	Georgia	*P. persica*	Positive, uredia produced
do.	*P. davidiana* Franch.	do.	do.	Positive, do.
March 12, 1936	*P. communis* Fritsch.	California	*P. persica*	Positive, do.
May 6, 1936	*P. serotina*	Arkansas	*P. serotina*	Positive, do.
May 13, 1936	do.	do.	*P. munsoniana*	Positive, do.
May 15, 1936	*P. armeniaca* L.	California	*P. persica*	Positive, do.
May 21, 1936	*P. communis*	do.	do.	Positive, do.
do.	do.	do.	*P. munsoniana*	Negative
do.	*P. munsoniana*	Arkansas	*P. persica*	Negative
do.	do.	do.	*P. munsoniana*	Positive, uredia produced
April 20, 1937	*P. persica*	California	*P. persica*	Positive, uredia produced
Sept. 14, 1937	*P. domestica* L.	do.	do.	Positive, do.
Sept. 29, 1937	do.	Oregon	do.	Positive, do.

leaves with aeciospores from these hosts. In 1931 Scott and Stout (28) reported the successful inoculation of various varieties of almond, apricot, cherry, nectarine, peach, plum, and prune, all cultivated species of *Prunus*, with aeciospores from *Anemone coronaria* L., a plant of European origin. They did not inoculate wild species of *Prunus*, but, in 1935, the writer obtained rust-infected *A. coronaria* plants[4] from California and inoculated the leaves of the peach and wild cherry, *Prunus serotina*, with aeciospores from this material (Table 2). Urediospores developed on the peach leaves but no infections were secured on the leaves of the wild cherry. These experiments were limited in number but confirmed Scott and Stout's results with respect to the peach and showed also that the aeciospores from *A. coronaria* will not infect the leaves of wild cherry.

Examination of Herbarium Material

The 389 collections (Table 3) of uredial and telial stages examined consisted of specimens of 26 species and 3 varieties of *Prunus*, both cultivated and wild, from various parts of the world, as well as a number of specimens in which the host was not identified.

No constant differences were noted between the uredial stages on the wild and cultivated species of *Prunus* in the United States. In general, the uredia are pulverulent, cinnamon-brown, and usually are formed on the dorsal surface of the leaves, although occasional specimens are encountered in which the sori occur on both surfaces. The urediospores are oblong-clavate or oblong-fusiform and measure 15–23 by 28–42 μ. The wall, which is 1 to 1.5 μ thick at the sides, is thickened at the apex. It is smooth and brownish-yellow at the apex but becomes echinulate and paler toward the base of the spore. The pores, which vary from 3 to 5 in number, may be equatorial but frequently, in the longer spores, are in the upper third. Capitate paraphyses are intermixed with the urediospores in the sori.

In contrast to the general uniformity of the urediospores produced on the various hosts the writer (10) found that the teliospores produced on the cultivated species can be distinguished readily, both macroscopically and microscopically, from the teliospores produced on the leaves of the wild species.

The teliospores (Fig. 1, A–E) produced on the wild species are bicellular, oblong or obovate-oblong, and measure 18–27 by 30–39 μ. The spores consist of 2 globoid or oblong-globoid cells, with chestnut-brown walls 1.5–2.5 μ thick, coarsely verrucose over the entire surface of both cells. Those (Fig. 1, F–I) produced by the fungus attacking the cultivated species are bicellular, dark chestnut-brown, with the upper cell globoid and the lower varying from globoid to irregular-shape forms, definitely contracted at the base. The wall of the upper cell is generally but not always thickened at the apex. It is much darker than the wall of the basal cell and is coarsely verrucose over its entire surface. The wall of the basal cell is never completely verrucose and, in some

[4] These plants were secured through the kindness of F. J. March and G. L. Stout, of the California State Department of Agriculture.

TABLE 2.—*Inoculation experiments with aeciospores of Tranzschelia pruni-spinosae at Fayetteville, Arkansas, 1935–36*

Date	Inoculum		Source	Host inoculated	Results
	Species	Host			
March 29, 1935	*Aecidium hepaticatum*	*Anemone caroliniana*	Arkansas	*Prunus serotina*	Positive, uredia produced
do.	do.	do.	do.	*P. persica*	Negative
April 6, 1935	do.	do.	do.	*P. serotina*	Positive, uredia produced
do.	do.	do.	do.	*P. persica*	Negative
April 12, 1935	*Aecidium punctatum* Pers.	*A. coronaria*	California	*P. persica*	Positive, uredia produced
do.	do.	do.	do.	*P. serotina*	Negative
April 30, 1935	do.	do.	do.	*P. persica*	Positive, uredia produced
April 26, 1936	*Aecidium hepaticatum*	*A. caroliniana*	Arkansas	*P. persica*	Negative
do.	do.	do.	do.	*P. serotina*	Positive, uredia produced
April 27, 1936	do.	do.	do.	*P. persica*	Negative
do.	do.	do.	do.	*P. munsoniana*	Positive, uredia & telia produced
do.	do.	do.	do.	*P. serotina*	Positive, uredia only
May 5, 1936	do.	do.	do.	*P. persica*	Negative
do.	do.	do.	do.	*P. serotina*	Positive, uredia only

TABLE 3.—*Enumeration of specimens examined arranged according to host species and geographical distribution. The first number opposite any species is the number of specimens on which teliospores were found; the second number indicates the number of specimens examined that did not have teliospores on them*

Host	North America	South America	Europe	Asia	Africa	Australasia
Prunus americana	23–0					
angustifolia	7–1					
armeniaca	2–8		3–1			0–1
" var. *anzu* Maxim.				1–0		
avium L.			1–0			
besseyi Bailey	5–1					
capuli Cav.		0–1				
cerasifera Ehrh.		1–0				
" var. *pissardii* Koehne			1–0			
chicasa Michx.	3–1					
communis	1–3		1–0	2–1		1–0
davidiana	3–2					
domestica	15–0		29–0			
" var. *insititia* Bailey			3–0			
grayana Maxim.				1–0		
hortulana Bailey	7–0					
japonica Thunb.		1–0				
mahaleb L.			2–0			
mexicana Wats.	3–0					
mume S. & Z.	2–0			1–0		
orthosepala Koehne	1–0					
pennsylvanica L.	2–0					
persica	14–93	0–19	1–4	0–3	2–2	3–3
pumila L.	3–0					
serotina	25–14					
simonii Carr.	1–0					
spinosa L.	1–0	1–0	16–0			
virginiana L.	4–1					
watsonii Sarg.	1–0					
sp.	20–5	2–1	5–1		2–0	

specimens, appears almost entirely smooth. The spores measure 15–23 by 26–39 μ. The basal cell generally is narrower than the apical cell.

Macroscopically the teliospores on the leaves of the wild species appear as pulverulent, chestnut-brown masses, while those on the cultivated species appear as compact, black clusters. The 2 types can be distinguished readily by these differences.

In 1901 Jacky (18) observed that the rust fungus attacking certain cultivated hosts in Germany produced teliospores that differ from those originally described by Persoon. Fischer (13) confirmed these observations and proposed that the teliospores produced by the form representing Persoon's species should be designated as the *forma typica* and the type discovered by

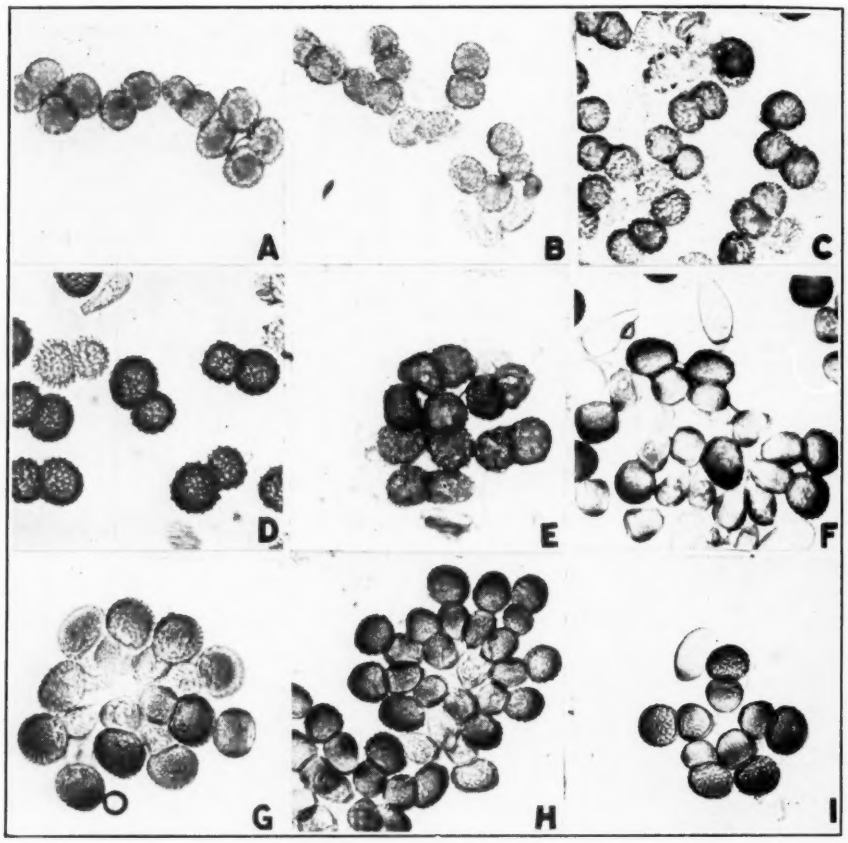

FIG. 1. Teliospores of *Tranzschelia pruni-spinosae* (Pers.) Diet., from the leaves of various species of *Prunus* in the United States. A–E. Examples of the variety *typica*. A. From *Prunus chicasa* Michx., Kansas, Sydow "Uredineen" 1179. B. From *P. besseyi* Bailey, Nebraska, J. M. Bates 451. C. From *P. hortulana* Bailey, Arkansas, Bartholomew "Fungi Columbiani" 2887. D. From *P. angustifolia* Marsh., Georgia, Dunegan 642. E. From *P. americana* Marsh., Texas, S. E. Wolff 198. F–I. Examples of the variety *discolor*. F. From *P. persica* S. & Z., Texas, Dunegan 875. G. From *P. domestica* L., California, D. W. Coquillet. H. From *P. davidiana* Franch., Georgia, Dunegan 835. I. From *P. mexicana* Wats., Georgia, Dunegan 834. All fixures ×270.

Jacky as the *forma discolor*. Subsequently, Kreig (19), Linsbauer (20), Eriksson (11), and Salmon and Ware (27) also recognized the occurrence of 2 types of teliospores in Europe. Grove (16), however, did not accept Fischer's proposals and stated that these differences in teliospores are ". . . hardly worthy of mention. . . ."

When the teliospores produced on the various species of *Prunus* in the United States were compared with European material, it was evident that those from the wild species in the United States were similar to the teliospores (Fig. 2, A–C) designated as the *forma typica* in Europe. The teliospores from the cultivated species in the United States were similar to those (Fig. 2, D–F) designated as the *forma discolor* in Europe. As a matter of conveni-

ence these 2 forms will be referred to in the following pages as the *typica* and *discolor* types, respectively.

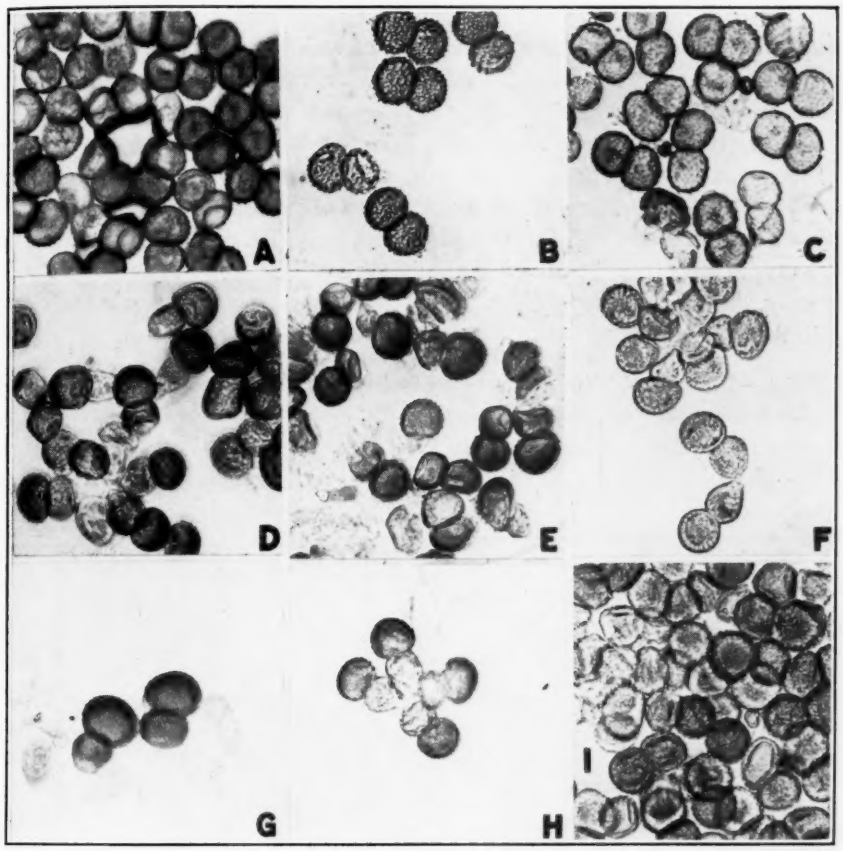

FIG. 2. Teliospores of *Tranzschelia pruni-spinosae* produced on various species of *Prunus* throughout the world, exclusive of United States. A–C. Examples of the variety *typica*. A. From *Prunus domestica*, Latvia, Kirulis "Fungi Latvici." B. From *P. domestica*, Austria, Petrak "Fungi Eichleriani" 192. C. From *P. spinosa*, Austria, Bresadola 7087. D–H. Examples of the variety *discolor*. D. From *P. domestica*, Italy, Bresadola. E. From *P. armeniaca*, Austria, de Thümen "Mycotheca Universalis" 1238. F. From *P. avium*, Spain, Caballero. G. From *P. communis*, Palestine, A. Aeronson "Fungi of Palestine" 60. H. From *P. spinosa*, Uruguay, G. Herter "Plantae Uruguayenses Exsiccatae" 1261. I. Teliospores from type material of Fuckel's *Puccinia discolor* (Fungi Rhenani 2121). All figures × 270.

In general, the *discolor* type of teliospore occurs almost entirely on the cultivated species, while the *typica* type predominates on the wild species; but, when the various specimens examined are arranged (Tables 4 and 5) according to the type of host (*i.e.* cultivated or wild), certain exceptions become evident. Thus some collections of *Prunus besseyi*, *P. hortulana*, and *P. mexicana*, wild species, had the *discolor* type teliospores on them. On the other hand, *P. armeniaca* and *P. domestica*, cultivated introduced species, were infected in a few instances by the form producing the *typica* type of

teliospores. Jacky (18) and others have observed a very similar situation. *Prunus spinosa* and *P. domestica,* normally infected in Europe with the form producing the *typica* type of teliospore, occasionally are infected with the *discolor* type. In the 29 *P. domestica* specimens of European origin examined by the writer, the *discolor* type of teliospore was found on 8 and the *typica* type on 21 specimens. Of the 15 *P. domestica* specimens examined from North America, the *typica* type of teliospore was found on only 2, while the *discolor* type was found on the remaining 13.

Since the genus *Prunus* is not indigenous in the Southern Hemisphere (except in the mountains of northern South America) the absence of the *typica* type in Africa and Australasia is not unexpected. The *discolor* and *typica* types were found on the few specimens available from Asia (Fig. 2, G), but only the *discolor* type (Fig. 2, H) was found on the specimens from South America.

TABLE 4.—*The type of teliospore found on various species of Prunus in North America*

Host	Type of host	Number of collections	Number of collections showing	
			discolor type teliospores	*typica* type teliospores
Prunus americana	wild	23	0	23
angustifolia	do.	8	0	7
armeniaca	cultivated	10	1	1
besseyi	wild	6	1	4
chicasa	do.	4	0	3
communis	cultivated	4	1	0
davidiana	do.	5	3	0
domestica	do.	15	13	2
hortulana	wild	7	1	6
mexicana	do.	3	2	1
mume	cultivated	2	2	0
orthosepala	wild	1	0	1
pennsylvanica	do.	2	0	2
persica	cultivated	107	14	0
pumila	wild	3	0	3
serotina	do.	39	0	25
simonii	do.	1	0	1
spinosa	cultivated	1	0	1
virginiana	wild	5	0	4
watsonii	do.	1	0	1
sp.		25	3	17

DISCUSSION

The results of the inoculation experiments and the study of the herbarium specimens clearly indicate that there are 2 distinct forms of the rust fungus attacking the genus *Prunus* throughout the world.

These results do not explain the variations noted in the prevalence and importance of the disease on the cultivated hosts. However, since the peach, as well as most of the other cultivated species, is infected only by the *discolor* type, these variations at least cannot be attributed to the existence of different species of rust fungi attacking the peach in various parts of the world.

TABLE 5.—*The type of teliospore of Tranzschelia pruni-spinosae found on species of Prunus in various parts of the world exclusive of North America*

Host	Type of host	South America		Europe		Asia		Africa		Australasia	
		discolor type	*typica* type	*discolor* type	*typica* type	*discolor* type	*typica* type	*discolor* type	*typica* type	*discolor* type	*typica* type
Prunus armeniaca	cultivated			2	1						
" var. *anzu*	do.					0	1				
avium	do.			1							
cerasifera	do.	1	0								
" var. *pissardii*	do.			0	1						
communis	do.			1	0	2	0			1	0
domestica	do.			8	21						
" var. *insititia*	do.			2	1						
grayana	wild					0	1				
japonica	cultivated	1	0								
mahaleb	do.			2	0						
mume	do.					0	1				
persica	do.			1	0						
spinosa	wild but with cultivated forms	1	0	1	15			2	0	3	0
sp.	——	2	0	2	5			2	0		

The demonstration of the existence of 2 forms confined to different host groups (*i.e.* cultivated and wild species) makes it advisable to modify the present concept of the species *Tranzschelia pruni-spinosae,* described in 1801 by Persoon (25), on the basis of a rust fungus he found on wild plum, *Prunus spinosa,* in Europe.

Although originally described from a wild host, the emended descriptions published by De Toni (30), Winter (32, p. 193), Arthur (3), and others, also include cultivated species as hosts of this fungus. The characters of the teliospores in these descriptions are clearly those of the *typica* type, and no description is given of the *discolor* type teliospore found on cultivated hosts.

Since the teliospores produced on wild species of *Prunus* in the United States are morphologically similar to those described by Persoon, it has been assumed that this form in the United States is identical with the European species. The *typica* type in the United States is associated with an aecial stage[5] on various native Ranunculaceae but, since there is now some confusion as to the aecial host of the *typica* type in Europe, the evidence that the form represented by the *typica* type in the United States is identical with Persoon's species rests solely on morphological characters of the teliospores. On the other hand, Kreig (19) proved in Europe that aeciospores of *Aecidium punctatum,* on *Anemone coronaria,* give rise to infections that produce the *discolor* type teliospores. This is the only type of teliospore found in the United States on the peach and allied species; and, when the experiments of Scott and Stout and the writer are recalled, it is clear that the successful infection of peach and other cultivated species was due to the presence of *A. punctatum* on cultivated *Anemone coronaria* in the United States. The fungus producing the *discolor* type teliospores on peach and other cultivated hosts in United States is, therefore, identical with the fungus producing the *discolor* type teliospores in Europe.

This type, however, has been demonstrated to be physiologically and morphologically distinct in the United States from the *typica* type. A name, therefore, should be applied to the *discolor* type to indicate this, for it is clearly not the fungus described by Persoon in 1801. Jacky (18) proposed the name *Puccinia discolor* Fckl., but, according to Winter (32, p. 193), Fuckel's fungus (14) is merely a variant of Persoon's species. The writer examined a portion of Fuckel's type material[6] and found that the teliospores (Fig. 2, I) were not the *discolor* type and Jacky's proposal is, therefore, not tenable.

To establish the *discolor* type as a new species would obscure the obvious close relationship existing between the 2 types. Furthermore, the specific name *pruni-spinosae* has been associated with the rust both on wild and cultivated species for at least 56 years. To end this long period of general

[5] Although *Aecidium hepaticatum* Schw. was originally described from *Hepatica acutiloba* DC. the writer considers the aecial stages on *Anemone quinquefolia* L. and *A. caroliniana* Walt. to be the same fungus, on the basis of the successful inoculation of various wild species of *Prunus* by several investigators in United States with aeciospores from these hosts.

[6] Fuckel, L. Fungi Rhenani exsiccati. No. 2121. 1867.

usage would only create confusion. This is especially true when it is realized that teliospores of the *discolor* type are produced so sporadically on some hosts[7] that routine collections made during most of the growing season could not be identified, as they would lack the spore form that is the criterion of the new species.

The writer, therefore, believes that the most logical procedure is to consider the 2 types as the varieties *typica* and *discolor,* respectively, of the variable species *Tranzschelia pruni-spinosae* (Pers.) Diet. A technical description embodying this disposition of the two types follows.

TRANZSCHELIA PRUNI-SPINOSAE

Aecidium punctatum Pers., Ann. Bot. Usteri 20: 135. 1796.
Puccinia Pruni-spinosae Pers., Syn. Fung. 226. 1801.
Puccinia Pruni DC., Fl. Fr. 2: 222. 1805.
Aecidium quadrifidum DC., in Poir. Encycl. Meth. Bot. 8: 235. 1808.
Puccinia Prunorum Link, in Willd. Sp. Pl. 6[2]: 82. 1825.
Uredo fusiformis Gachet, Act. Soc. Linn. Bordeaux 5: 232. 1832.
Aecidium hepaticatum Schw., Trans. Am. Phil. Soc. 11. 4: 293. 1832.
Uredo Pruni Cast., Obs. 1: 27. 1842.
Uromyces Prunorum Fckl., Jahrb. Ver. Nat. Nass. 15: 20. 1861.
Dicaeoma Prunorum Rabh., Fungi Eur. 990. 1866.
Puccinia discolor Fckl., Fungi Rhenani 2121. 1867.
Dicaeoma Pruni-spinosae Kuntze, Rev. Gen. Pl. 3: 470. 1898.
Aecidium dakotensis Griff., Bull. Torrey Club 29: 300. 1902.
Aecidium Aikeni Sydow, Ann. Myc. 1: 334. 1903.
Tranzschelia punctata Arth., Res. Sci. Cong. Bot. Vienne 340. 1906.
Tranzschelia pruni spinosae (Pers.) Diet., Ann. Myc. 20: 31. 1922.

Pycnia chiefly epiphyllous, evenly and remotely scattered, conspicuous, aecia hypophyllous, scattered, cupulate, dehiscent into few (often 4) widely spreading lobes; aeciospores globoid or oblong-globoid, 15–23 by 18–26 μ; wall cinnamon-brown, 1.5–2.5 μ thick, verrucose.

On Ranunculaceae: The species are listed under the two varieties.

Uredia hypophyllous, cinnamon-brown, pulverulent; urediospores oblong-clavate or oblong-fusiform, 15–23 μ by 28–42 μ, with capitate paraphyses intermixed; wall brownish-yellow above, paler below, 1–1.5 μ thick at sides, 5–9 μ above, smooth above, sharply echinulate below, the pores 3–5, equatorial. Telia hypophyllous, dark chestnut-brown, somewhat pulverulent; teliospores oblong or obovate-oblong, 18–27 by 30–39 μ, the two cells globoid or oblong-globoid and easily separable; the basal cell frequently smaller than the apical cell; wall chestnut brown, often paler in the basal cell, usually 1.5–2.5 μ thick but frequently thickened at top of apical cell, coarsely verrucose over both cells or the basal cell only sparsely so; pedicel colorless, readily detached from the spore.

On Amygdalaceae: The host species are listed under the 2 varieties.

Range: A cosmopolitan species, occurring throughout the world.

The rust fungus on the genus *Prunus* is a variable species and can be separated into two varieties according to the combination of aecial and telial hosts, as established by inoculation experiments, and by a difference in the morphology of the teliospores.

(1) **Tranzschelia pruni-spinosae typica** n. comb. (*T. pruni-spinosae forma typica* Fischer).

[7] For example, Scribner (29) stated in 1887 that he had never found teliospores on the peach and thought it was probable that they were not formed on this host. This is not the case, although they were found on only 20 of the 144 peach specimens examined by the writer.

Pycnia and aecia as above.

On Ranunculaceae: Anemone caroliniana Walt., *A. quinquefolia* L., *Hepatica acutiloba* DC. (*H. acuta* Britt.), *H. triloba* Chaix. (*H. hepatica* Karst.), *Ranunculus recurvatus* Poir., *Thalictrum dasycarpum* Fisch. and Lall. (*T. purpurascens* Arth., not L.), *T. dioicum* L., *T. polygamun* Muhl. (*T. cornuti* T. and G.) in North America; *Anemone ranunculoides* L. in Europe; and probably also in Asia on wild species.

Urediospores as above; teliospores with both cells of approximately the same size and color, the wall not noticeably thickened at top of the apical cell and uniformly coarsely verrucose over both cells.

On Amygdalaceae: Reported on various wild species of *Prunus* in North America, Europe and Asia, on the cultivated species *Prunus armeniaca* L., *P. cerasifera* var. *pissardii* Koehne, *P. domestica* L., *P. domestica* var. *insititia* Bailey, and *P. mume* S. & Z., in Europe and Asia.

Range: Throughout the Northern Hemisphere zone, wherever wild species of *Prunus* occur, and possibly extending into the Southern Hemisphere on species of *Prunus* growing in the Andes Mountains. Although morphologically similar forms of this variety occur in various hosts in many parts of the world, it is assumed only that they are identical. No inoculation experiments have been performed to confirm this assumption.

(2) **Tranzschelia pruni-spinosae discolor** n. comb. (*T. pruni-spinosae forma discolor* Fischer).

Pycnia and aecia as above.

On Ranunculaceae: Anemone coronaria L. in Europe as an indigenous species, elsewhere as a cultivated introduced plant. Reported definitely from the United States, Australia, and New Zealand.

Urediospores as above; telia scattered, generally produced sparsely except on *Prunus domestica* L., teliospores distinguishable from those of *T. pruni-spinosae typica* by the fact that the wall of the apical cell is uniformly and coarsely verrucose, while that of the basal cell is sparsely, if at all, verrucose. The apical cell is globoid with the wall frequently thickened at apex. The basal cell is oblong or obovate-oblong, generally narrowed toward base and lighter in color than the apical cell.

On Amygdalaceae: Prunus armeniaca, North America, Europe, Asia, and Australasia.—*P. avium*, Europe.—*P. besseyi*, North America.—*P. cerasifera*, South America.—*P. cerasifera* var. *divaricata* Ledeb., Europe.—*P. communis*, North America, Europe, Asia, Australasia.—*P. davidiana*, North America.—*P. domestica*, North America, Europe.—*P. hortulana*, North America.—*P. japonica*, South America.—*P. mahaleb*, Europe.—*P. mexicana*, North Ameria.—*P. mume*, North America.—*P. persica*, North America, South America, Europe, Asia, Africa and Australasia.—*P. spinosa*, South America, Europe.

Range: Throughout the world, wherever cultivated species of *Prunus* are grown. Found also on cultivated forms of some wild species.

The 3 species of *Thalictrum* and *Ranunculus recurvatus* Poir., are included, on the authority of Arthur, as aecial hosts of the *typica* variety in the

United States. The inclusion of *Anemone ranunculoides* as the European host of the same variety is based upon statements to this effect in the literature. The writer has not had an opportunity to examine aecial material on any of these hosts nor to use aeciospores from them in inoculation experiments.

The list of uredial and telial host plants for the 2 varieties is considered incomplete, but specimens of all those listed, except *Prunus cerasifera* var. *divaricata*, have been examined and the variety of rust fungus present on them identified.

The urediospores of both varieties show wide variation in size and shape, and no constant character was noted that would distinguish the urediospores of one variety from those of the other. Cristinzio (7), however, reported that he could distinguish between the urediospores produced on a number of cultivated hosts on the basis of size of spores and shape of the paraphyses. Since his cross-inoculation experiments showed no genetic variations that corresponded with the observed morphologic differences, it is evident that all the hosts (almond, apricot, plum and peach) were infected by the same variety. The differences he observed, therefore, are not differences between the urediospores of the varieties *typica* and *discolor* but are merely variations within the variety *discolor*.

Finally, it should be mentioned that *Eranthis hiemalis* (L.) Salisb. is not a host plant for either of the varieties although frequently considered as such in Europe. Tranzschel (31) has recently demonstrated that the fungus on this plant is the aecial stage of *Puccinia cerasi* (Bereng.) Cast., an entirely different rust from the one discussed in this paper. He has created a new genus *Leucotelium* to which he has transferred *Puccinia cerasi* (Bereng.) Cast. and *P. pruni-persicae* Hori. The teliospores of these rusts are two-celled, smooth-walled, hyaline spores quite different in appearance from the rough-walled, colored teliospores of *Tranzschelia*.

SUMMARY

The rust fungus commonly known as *Tranzschelia pruni-spinosae* (Pers.) Diet. is the cause of a disease of considerable economic importance on *Prunus* spp. throughout the world.

The disease is confined primarily to the leaves, but, under certain conditions, has been known to attack the fruit of the peach. Marked variations have been noted in the prevalence and importance of the disease.

In a study of the causes of these variations it was found that urediospores from cultivated hosts from various parts of the United States would infect peach leaves but would not produce infections on the leaves of wild species of *Prunus*. Conversely, urediospores from various wild species would not infect the leaves of the peach. Likewise, aeciospores from wild species of *Anemone* would not infect peach leaves but would infect leaves of wild species of *Prunus*, whereas aeciospores from a cultivated species of *Anemone* would infect peach leaves but would not infect those of wild species.

An examination of 389 herbarium specimens showed that the teliospores produced on the leaves of the cultivated species are morphologically different from those produced on the leaves of the wild species of *Prunus*.

These results, while they do not explain the variations noted in the prevalence and importance of the disease, do show that these variations are not due to occurrence of different species of the rust fungus on the cultivated species of *Prunus*, for the same type of teliospores was found on them throughout the world almost without exception.

The demonstration of the existence of 2 forms of rust fungus confined to different host groups (*i.e.* cultivated and wild species) in the genus *Prunus* necessitates a revision of the present concept of Persoon's original species *Tranzschelia pruni-spinosae*. This species is considered separable into 2 varieties according to the combination of aecial and telial hosts and differences in the morphology of the teliospores. The combination *Tranzschelia pruni-spinosae typica* is proposed for the variety found on wild species and *T. pruni-spinosae discolor* for the variety attacking the cultivated species. Technical descriptions of these new combinations are included in the text.

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A BACTERIOSIS OF DAHLIA, ERWINIA CYTOLYTICA

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INTRODUCTION

Very little thorough work has been done on the stem rots and blights of dahlia. In 1933 the late Dr. M. A. Howe observed a stem rot of dahlia then present in the New York Botanical Garden. Reference to this fact was made by M. T. Cook (2), and the fungus associated with the trouble was identified as *Sclerotinia libertiana* (Lib.) Mass. The disease in the Garden was confined to the Snowdrift and Shasta varieties.

As described by Cook, the first evidence of the disease is a water-soaked appearance of the stem, accompanied by wilting. Finally, the diseased parts die, and become black and dry. The central cavity of the stem is more or less filled with a luxuriant growth of mycelium, accompanied by the appearance of sclerotia. Beyond the identification of the fungus no experiments were undertaken, and its pathogenicity was not established.

Since then, the occurrence of the fungus on dahlia in other parts of the country has been recorded in the plant-disease surveys of the U. S. Department of Agriculture. It should be noted, however, that in the disease under

[1] Grateful acknowledgment is given by the writer to Dr. B. O. Dodge for assistance and advice and for the facilities of his laboratory.

discussion no such condition exists and no such fungus growth occurs in the decayed pith tissue.

In 1933, under the title: A New Disease of Dahlias, Thelma B. Post (5) of the Bureau of Plant Industry, U. S. Dept. of Agriculture, described a stem blight of dahlia with which a fungus was associated, identified as *Macrophomina phaseoli* (Maubl.) Ashby.

To quote the author: "Most of the stem tissue was blackened, but some water-soaked tissue was still evident. The pycnidia occurred in great numbers in the epidermis of the blackened areas. As the stem tissue dries the fibres separate easily and the ends are split and frayed. Most of the pith disappears leaving only a thin brittle remnant in which the sclerotia are embedded in such profusion that it had the appearance of a black crust." Cultures of this fungus from single pycnidiospores produced both sclerotia and pycnidia; or again, only sclerotia, previously identified as *Rhizoctonia bataticola* (Taub.) Butl., thus establishing the identity of the two stages. However, no plant inoculations were made to establish the pathogenicity of the fungus. Whether this organism is the same as that noted by Cook is an open question. However, in the disease under consideration no pycnidia developed on the epidermis, nor were there sclerotia in the pith tissue in specimens kept for a long time in a moist chamber.

In 1927[2] mention is made of a Fusarium wilt of dahlia occurring in the District of Columbia and in Missouri, but no studies were made of the disease at the time.

J. F. Adams (1), in 1927, makes repeated reference to the "Stunt" or "Dwarf" disease of dahlia, but was unable to attribute it to any single cause.

The first reference to bacteria pathogenic to dahlia is recorded by Hori (4), who described a wilt that attacks both stems and tubers, and the causal organism *Bacillus dahliae*. Hori's paper is not available, but Elliott (3) gives a brief description of the bacillus and the symptoms of the disease. According to Elliott, the organism belongs to the genus *Erwinia*, although the available data are insufficient to correlate it with the present organism, even though the symptoms are similar.

In 1922 Wolf (6) isolated from the xylem tissue of dahlia a bacterium that he considered the same as *Phytomonas solanacearum* (Erw. Smith) Bergey *et al.*, ordinarily known as the cause of brown rot of Solanaceae. Wolf made no inoculations; hence, we must reserve opinion as to its pathogenicity for dahlia. Realizing the importance of Wolf's work, however, we attempted to find this organism in our own material, but were unsuccessful and, therefore, concluded that the bacterial trouble under consideration was of different origin. Since then, the occurrence of a bacterial wilt of dahlia has been reported, attributable to *Phytomonas solanacearum*, apparently on the assumption that it was the same as that reported by Wolf.

[2] Martin, G. H. Diseases of forest and shade trees, ornamental and miscellaneous plants in the United States in 1926. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. Sup. 55. 1927. [Mimeographed.] (See p. 365.)

BACTERIOSIS OF DAHLIA

In late August, 1936, attention was called to a stem rot of dahlia in the Garden. This disease becomes externally recognizable through a blackening and softening of the stem. Stems cut open lengthwise show a moist and advanced decay of the layer of pith lining the hollow stem, whence it extends into the cortex, which becomes blackened. The decaying stems swarm with bacteria and give off a foul odor not unlike that of mice.

To isolate the bacteria, an infusion of the decayed tissue was made in sterile water from which beef-peptone-agar plates were poured, which, in high dilutions, gave numerous colonies, mostly of a prevailing type, and frequently also, a few colonies of a larger spore-forming bacillus, apparently not a pathogen.

The former prevailing organism gave colonies (Cult. Dal. 2. Table 1) of a distinctive appearance, which were subsequently easily recognized in other isolations. In two days at 25°–30° C., they average about 2 mm. in diameter, are circular, convex, watery, glistening, pale grayish, and translucent. By transmitted light they are strongly refracting, pale drab to light brownish-yellow, nearly or quite amorphous, with sharply defined, entire borders. The bacteria are actively motile, short rods averaging 0.7×1.5–$4\ \mu$, occurring singly and in pairs. The flagella are peritrichous, thus definitely placing it in the genus *Erwinia*.

To determine whether this organism (Dal. 2), which we shall designate as the original isolation, is pathogenic to dahlia, sections of healthy stems were washed clean in running water, then placed for 5 minutes in 1:1000 $HgCl_2$ solution, the latter removed by rinsing with sterile water. The stems were then placed in sterile Petri dishes and inoculated by puncture with a pure culture of the organism in question. Drying out was prevented by placing the dishes under a bell glass. Checks were made by punctures with a sterile needle.

Generally, within 2 days, a decided decay had set in from the points of inoculation, which continued to extend into the pith. Bacteria were abundant. To compare these with those used as inoculum, plate cultures were grown upon beef-peptone agar. In all cases there developed numerous colonies of the type already described. Transfers were made from these to agar and to different media to establish the identity of the separated organism with the original isolation. These inoculations were repeated in sufficient number to confirm previous tests regarding the pathogenicity of the organism.

The occasional presence of the aforementioned sporulating *Bacillus*, which seemed to be the same species throughout, raised the question whether it might not also be pathogenic or at least contribute to the decay. Accordingly, stems were inoculated with a pure culture of the latter spore-forming organism. No infection resulted in any case, while stems inoculated simultaneously with the original isolation (Dal. 2) showed advanced decay within 3 days. The same decay resulted from inoculations with a mixed suspension

of the two organisms showing at least that the presence of the sporulating *Bacillus* did not inhibit the infection of the other.

In bacteriosis of the dahlia, the decay generally starts at the crown, indicating that the original infection comes from the soil, although this is not always accompanied by any marked decay of the tuber. The bacteria at first make slow progress in the cortex, and the more rapid growth in the pith tissue is due partly to its parenchymatous character and perhaps also to the presence of air in the hollow stem. When, however, the pith becomes thoroughly decayed, soft, and watery, the bacteria rapidly invade the outer layers, when bacteria are present in great numbers. As soon as the outer ring of woody tissue becomes involved, it results in complete softening and disintegration, the coherence of the woody fibres is destroyed because of the intracellular dissolution.

Whenever tuber decay accompanies that of the stem, we would expect to find the same organism in the tuber. That the same is true is shown by our isolates in series IX, table 1.

TABLE 1.—*Results obtained from artificial inoculation of dahlia stems with Bacillus, Dal. 2, from cultures grown in colony plates and inoculated stems*

Ser.	Cult.	Inoculant	Source of culture	Group number
I	Dal. 2	Decayed stem		5010.32120.1222.
I	Dal. 2–5	Dal. 2		2201.00.211.22.100.0
III	Dal. 2–1	Dal. 2	Colony Plate Inoc. Stem	The other cultures in the table have the same group numbers.
III	Dal. 24–2	Decayed pith	do	
V	Dal. 2–A–1	Dal. 2	do	
VI	Dal. 20–2	Dal. 2	do	Group numbers according to descriptive chart. Soc. Am. Bacteriologists, Dec. 30, 1929.
VII	Dal. 18–2	Dal. 2		
VIII	Dal. 23–2	Dal. 2	do	
VIII	Dal. 19–1	Mixed cult Dal. 2 and Fusarium	do do	
IX	Dal. 29–1		Decayed tuber	
X	Dal. 2		do	

Slices of tubers also were inoculated with a pure culture of the original isolate (Dal. 2), with the result that decay followed within 3 days.

Table 1 shows the main cultural and physiological characteristics of the organisms isolated from naturally decayed dahlia stems and tubers, together with those isolated from inoculated material.

Series I is the original isolation from a decayed stem, Series IX the same isolation from a decayed tuber. Both organisms are the same. Series X is an isolation from tuber sections inoculated with the original isolate which showed advanced decay within 3 days; also the same. The other isolate, series III, V, VI, VII and VIII, were from decayed stems, inoculated with the original isolate.

THE ORGANISM

The present organism does not agree with any of the recorded species in the genus *Erwinia*, hence, it is regarded as new and the name **Erwinia cytolytica** is proposed. The following is a description of its cultural and physiological characteristics.

Morphology. From cultures grown on beef-peptone agar, filmed, fixed in the flame, and stained with carbol-fuchsin, the vegetative cells average 0.6–0.7 μ in diameter by 2.5–3.5 μ in length, with occasionally longer forms up to 5.0 μ. Fixed with Loffler's mordant and stained with carbol fuchsin, they are broader, up to 1.0 μ, indicating the presence of a capsule that also could be demonstrated by the usual methods of staining. They occur singly and in pairs, and are gram-negative. Stained by the Van Ermingen method, the flagella are extremely delicate and arise from any part of the body of the cell. They are, therefore, peritrichous.

Spores. Absent. Broth, inoculated with a fresh culture, gave no growth when heated in water bath for 15 minutes at 75° C., while nonheated check tubes gave good growth.

Agar Colonies. On beef-peptone agar pH 7.2, 2 days at 25°–30°, 2–3 mm. diameter, round, convex, moist, glistening, grayish white, watery, translucent; by transmitted light, low magnification, light brownish yellow, amorphous; borders distinct, entire, undulate.

Agar Slant. Same medium as above. Growth thin, nearly white, translucent, smooth, moist, glistening, butyrous. Medium not discolored. The growth is more profuse on the same medium containing dextrose.

Beef-peptone Broth. pH 7.2. A uniform turbidity, no surface growth, flocculent sediment.

Gelatin Stab. Medium slowly liquefied. At first, a crateriform depression, becoming infundibuliform in 7 days at 20° C.

Potato. Growth light brown, moist, glistening, smooth or widely contoured, medium not distinctly darkened.

Litmus Milk. Coagulated in 5–7 days at 25°–30° C., slightly acid; not digested; litmus reduced.

PHYSIOLOGICAL CHARACTERS

Aerobic and facultative anaerobic. In fermentation tubes of dextrose and sucrose broths, growth in closed arm; nitrates reduced to nitrites, indol-negative; H_2S not formed.

Starch hydrolyzed; acetylmethyl carbinol, a slight reaction over night. Ammonia production in beef-peptone broth, negative, only a slight increase in alkalinity.

Fermi's solution, good growth; Cohn's solution, no growth. No invertase in sucrose broth. In synthetic media containing mineral salts and ammonium phosphate as a source of nitrogen, acid produced, without gas, from dextrose, lactose, sucrose, raffinose, mannite, salicin, isodulcite. No acid from levulose, arabinose, xylose, glycerose, inulin. In synthetic media, as before, with dextrose as a source of carbon, good growth with asparagin, peptone, and ammonia as a source of nitrogen. No growth from KNO_3 as a source of nitrogen.

Pectinase Production. To test the production of this enzyme a fresh broth culture of the organism was filtered through a sterilized thimble of unglazed porcelain into a sterile flask. To the filtrate 0.5 per cent of thymol was added, and then distributed into sterile test-tubes. All these tubes remained sterile.

Thin slabs, under asceptic conditions were cut from a raw dahlia tuber, and transferred to tubes containing the sterile filtrate. A few of these showed contamination; the others remained sterile and clear.

Slabs in the latter tubes began to show in a few days signs of softening and dissolution, which increased until, on shaking, the tissue fell apart into a soft mushy mass.

Other tubes similarly treated were heated for 10 minutes in a boiling water bath. The slabs in these tubes remained intact. No effort was made to precipitate the enzyme.

Temperature relations. Optimum temperature 28°–30° C. Grows at 37° C. Slow growth at 20°, no growth at 8°–10° C.

Relations of Media. Approximately equal growths in beef-peptone broth at pH 6.8 to 7.3. Feeble growth at pH 5.0. No growth at 4.4.

SUMMARY

A stem rot of dahlia, which also affects the tubers, produced by a bacterium considered new and named *Erwinia cytolytica*. The organism has been isolated from decayed stems and tubers and its characteristics determined. Inoculations of pure cultures into dahlia stems and tubers gave the characteristic soft rot and from which the same organism was recovered.

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A ROOT ROT OF PEAS CAUSED BY FUSARIUM COERULEUM

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(Accepted for publication February 17, 1938)

The pea, *Pisum sativum* L., root-rot complex is composed of a number of different soil-inhabiting fungi that cause symptoms so interrelated that they cannot be identified with certainty by a macroscopic examination. Several of these fungi, widely separated in relationship, may occur in the same field, and more than one have been found to parasitize the same plant. The various organisms and the diseases they cause have been subjects of study by different investigators, both in the United States and in foreign countries (3), for a number of years, with the result that new parasites have been added to the list from time to time. The literature on pea diseases and in particular on the root rots is so extensive that no attempt will be made to review all or any considerable part of it.

In 1936, Geach (4) published an article on the root rot of grey peas that he thought was caused probably by a strain of *Aphanomyces euteiches* Drechsler, and, a few years earlier, Brown and Evans (1) described two diseases of peas new to Arizona, one of which, a species of the genus *Fusarium*, had never been associated previously with any disease of the crop. Other species of *Fusarium* have been isolated from peas and some of them proved to cause extensive damage to the crop. Jones (8) in 1923, published the results of his investigations on the stem and root rot of peas in which he showed that a new variety of *F. martii* that he described as *pisi* caused extensive losses in various parts of the United States. While species of *Fusarium* are known to cause extensive root-rot losses, the greatest damage is believed to be caused by members of the Phycomycetes (2, 4, 5, 6, 7, 9), among which may be mentioned species of *Pythium* and *Aphanomyces*. Their known distribution over the world is probably more extensive than that of any other root-rot fungus.

From several field surveys of pea diseases in the United States, the writer isolated with considerable frequency a species of *Fusarium* among other organisms that proved to be different from any heretofore described as a cause of pea root rot. Since it causes symptoms somewhat resembling those caused by *Fusarium martii* App. and Wr. var. *pisi* Jones, it probably was long overlooked as a possible parasite. While culturally and morphologically it was different from *Fusarium martii pisi*, there was sufficient resemblance from a casual examination to be mistaken for it. However, a more critical study of the organism showed that it was definitely a different species of *Fusarium*. In view of this fact experiments were undertaken to determine if it caused root rot of peas or was merely another saprophytic invader of dead plant tissue. This article discusses the results of the investigations with the organism and the disease it causes.

ECONOMIC IMPORTANCE AND DISTRIBUTION

There is no way of estimating, even approximately, the losses caused by this disease. It is induced by only one of several organisms that cause root rots of peas and occurs along with others in the field. The causative organism is one of those fungi that may or may not kill the plant but that retard its growth and consequently reduce the yield. The degree of resultant damage naturally depends on the age of the plant when attacked by the parasite and the kind of weather that follows.

To determine its distribution is not so difficult, although it is not entirely known. The causal organism has been isolated from the roots of pea plants in Maryland, Virginia, Colorado, Idaho, and Maine, and its distribution may be coextensive with that of the pea. Like other root-rot organisms, it is most abundant in those fields that have been long under cultivation and especially those fields planted frequently to peas.

SYMPTOMATOLOGY

Symptoms of a root rot under field conditions where there is competition with different pathogenic organisms are difficult to describe. The clearest

description can be made from plants infected in sterilized and, later, inoculated soil.

Under these conditions the causal fungus is particularly aggressive. Many of the seeds are rotted before they germinate and many of those that germinate are destroyed before the plant emerges through the soil, or soon thereafter, by decay the tap root, thereby leaving only a stub at the region of the attachment of the root to the seed. If, however, the plant escapes early decay, the first symptoms appear on the tap root in the region of its attachment to the seed and consist of small, black spots and streaks that are first observed about 3 weeks after planting the seed. As the plant grows, the infection areas extend up, down, and around the tap root (Fig. 1, A) to the soil line, involving most of the cortex. In the later stages of the disease some of the lateral roots (Fig. 1, B) become infected. The plant is rarely killed if it attains a height of 6 or 8 inches before it is attacked by the fungus.

Many of the symptoms of this disease are characteristic of the root rot of peas shown by Jones (9) to be caused by *Fusarium martii* var. *pisi*. The only sure way to determine which organism is causing the disease under field conditions is to make isolations and identify the fungus.

ETIOLOGY OF THE DISEASE

The causal organism is a species of *Fusarium* belonging to the section *Martiella*. While the spore sizes of the different isolates studied do not entirely agree with those given by Wollenweber and Reinking (10) for *Fusarium coeruleum* (Lib.) Sacc., the fungus agrees culturally and morphologically more nearly with that species than with any other. There is considerable variation in spore sizes, which average larger than those generally given for the species. The writer, however, believes that if it is not identical with *F. coeruleum* there is no more than a variety difference.

PATHOGENICITY

Many different fungi, among them several species of *Fusarium*, have been isolated from the underground parts of pea plants. Not all of them were parasitic and most of them were eliminated by inoculation tests or were identified as species previously proved to be root rot producing fungi. Those that showed any promise of being parasites were tested by additional inoculations. Among the organisms tested in an exploratory way was *F. coeruleum*, which proved to be a vigorous parasite; in fact, even more so than *F. martii* var. *pisi*. In view of these facts controlled inoculation experiments were carried out with different isolates. All the isolates were obtained from the roots of peas except one, which was furnished the writer by Freeman Weiss who isolated it from potatoes held for some time in storage.

A few preliminary experiments were conducted in the greenhouse to determine the parasitism of the causal organism in sand cultures. The sand was sterilized by heating 5 hours at about 10 pounds' pressure. Oats on

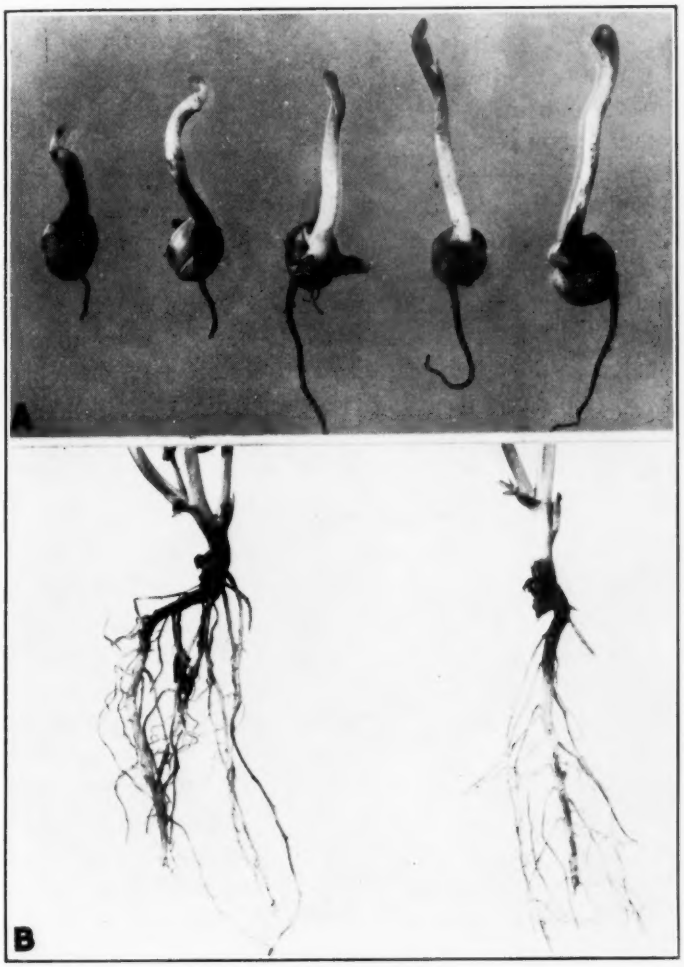

FIG. 1. Pea root rot caused by *Fusarium coeruleum*. A. Various types and degrees of infection of seedlings prior to or at time of emergence from soil. In all cases, cortex of tap root was destroyed. The pathogen already had attacked the stem in some cases and not in others. B. Two plants attacked when about 4 weeks old. Causal organism has extended up to the stem almost to surface of soil and downward onto tap root and rootlets.

which *Fusarium coeruleum* had grown for about 2 weeks were worked into the sand and 2 days later pea seed (Little Marvel and Harrison Glory varieties) was planted. Seven days later a few plants came up, but a careful examination later showed that most of the seed or plants had been destroyed before they emerged through the sand. Duplicate preliminary experiments gave similar results. These experiments were supplemented by the use of sterilized soil. A culture of the organism was grown on oats and stirred into the soil and 2 days later 400 seeds each of Little Marvel and Harrison Glory were planted. One hundred seeds of each variety were planted, as controls, on the noninoculated soil. In these experiments most of the plants came up and grew fairly well. At the end of 4 weeks they had attained a height of

a foot or more, when they were removed from the soil, washed, and examined for root-rot lesions. At that time 100 per cent of the plants were diseased, and most of them showed a conspicuous blackening of the cortex on the lower part of the stem and on the tap root and some laterals. None of the controls were diseased. They were longer and better plants than those in infested soil, showing that, although the vines looked healthy, growth was retarded. A duplicate planting was made on the same bed of soil immediately on removal of the first crop without the addition of any more inoculum. The second experiment gave almost identical results. All the plants became infected, but not so early as when the seed was sown 2 days after mixing the inoculum into the soil. It is interesting to note in connection with these experiments that, although the cortex was badly diseased, the foliage showed little evidence of any root trouble. Because of the frequent watering under greenhouse conditions, the plants evidently withstood the disease much better than they would had they been grown under normal field conditions.

VARIETY TEST

Twenty-four varieties were selected for testing in soil that had been steam-sterilized 7 hours on 3 consecutive days at about 10 pounds' pressure. The soil was spread on a bench in the greenhouse and inoculated with oat cultures, as described previously. Two days after the soil was inoculated, one half of it was planted, using 25 seeds of each variety. The Little Marvel, previously shown to be susceptible, was used as a susceptible control on non-inoculated soil. The second planting was made 7 days later. Only a small percentage of the seed of the first planting came up and the resultant seedlings were badly diseased and died soon thereafter. Most of them were killed before they emerged from the soil. The second planting did much better. More of the seed came up and the young plants grew well. In about 18 days after planting the cortex of the lower stem and of the tap-root turned black. The plants were removed from the soil and examined for root rot at the end of 30 days with the results shown in table 1.

An examination of table 1 shows that none of the varieties are immune from the disease; in fact, it is doubtful if any of them could be classed as tolerant. The probabilities are that those varieties in which some plants were only slightly or moderately diseased, escaped infection for a time and, consequently, did not show the same degree of injury when the experiment was terminated. No resistant varieties were discovered by these experiments. It is, however, interesting to note that, under the conditions of these tests, the parasite did not destroy the germinating seed and seedlings so actively after it had been in the soil for a week or more as it did when first introduced.

The pigeon pea, *Cajanus indicus,* was almost completely immune.

TEMPERATURE RELATIONSHIP

Several attempts were made to fix the cardinal temperatures for the growth of the organism in conjunction with the host by means of tempera-

TABLE 1.—*Susceptibility of 24 varieties of peas to the pea root-rot organism, Fusarium coeruleum.* (*25 seeds planted*)

Variety	Number of plants to emerge	Degree of infection
Pisum sativum		
Alaska	15	3 moderate, 12 severe
Alderman	12	12 severe
Asgrow Alaska	14	14 "
Blue Bell	11	11 "
Canners Perfection	18	18 "
Capucijner	22	22 "
Daisy (Dwarf Telephone)	14	14 "
Horsford	16	6 slight, 10 moderate
Hundredfold	15	15 severe
Large Podded Alaska	16	1 slight, 15 severe
Laxtons Progress	12	12 severe
Little Marvel	7	7 "
Onward	13	13 "
Perfection	10	10 "
Pioneer	21	21 "
Potlatch	3	3 "
Stratagem	12	12 "
Surprise	8	8 "
Telephone	12	5 slight, 4 moderate, 3 severe
Thomas Laxton	17	17 severe
World Record	20	20 "
Pisum sativum arvense		
Austrian Winter	24	24 severe
Maple	25	25 "
Victoria	20	20 "

ture-controlled soil tanks. Briefly, the method consisted in the use of sterilized soil into which was mixed oat cultures of the fungus. Seed of the varieties to be tested was planted a day or two later. The temperatures covered a range from 10° to 40° C. at 5° intervals. Many of the seeds were destroyed as soon as germination started and only a few emerged from the soil. Almost no plant growth occurred at the low and high temperatures. The soil was prepared to contain 35 per cent of its water-holding capacity and maintained at that amount by adding water daily to bring it to weight. The soil contained sufficient moisture for seed germination and plant growth. The failure of the above described method suggested the possibility of germinating the seed in pure sand and then transplanting to the soil tanks after a copious root system had been developed. The plants did not survive transplanting at the extreme temperatures and at intermediate temperatures recovery was very slow. Lacking the vigor of thriving plants they soon succumbed to the attacks of the fungus. An analysis of the data obtained by these experiments allows only one conclusion to be drawn, *i.e.*, the causal organism has a temperature range of parasitism that corresponds to the temperatures at which the host will grow.

SUMMARY

A root rot caused by *Fusarium coeruleum*, not previously recognized as a parasite of peas, has been discussed. The causal organism is shown to be

a vigorous parasite, and widely distributed in the United States.

Twenty-four varieties of peas were planted in artificially infested soil and no resistance or tolerance was shown by any of them. Pigeon pea, *Cajanus indicus*, is immune.

The temperature range of the pathogen corresponds to that of the temperature permitting growth of the host.

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EYE-SPOT DISEASE OF NAPIER GRASS

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(Accepted for publication February 10, 1938)

In 1935, Leukel and Camp[1] reported that the yields of Napier grass, *Pennisetum purpureum* Schum., in their fertility plots at Gainesville, Florida, were severely affected by a disease, and that selections were being made for resistance from the few plants remaining healthy. Later, Stokes and Ritchey[2] reported that the disease was caused by *Helminthosporium ocellum* Faris, and that resistant strains of the grass had been found, the organism being identified by the writer. In 1928, *H. ocellum* was reported by Faris[3] as causing eye spot of sugar cane in Cuba, Puerto Rico, and Santo Domingo. In 1934, Bourne[4] also proved that this fungus was the organism causing eye

[1] Leukel, W. A., and J. P. Camp. Fertilization of pasture and forage grasses. Fla. Agr. Exp. Station Ann. Rpt. 1934–35: 41. [1935?].

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[3] Faris, James A. Three Helminthosporium diseases of sugar cane. Phytopath. 18: 753–774. 1928.

[4] Bourne, B. A. Studies on the ring spot disease of sugar cane. Fla. Agr. Exp. Sta. Bull. 267. 1934.

spot of sugar cane in Florida, and that when accompanied by such secondary organisms as *Phyllosticta sorghina, Nigrospora* sp. and *Leptosphaeria sacchari,* a typical ring-spot disease is produced. No typical ring spots were observed on the Napier grass at Gainesville, although sugar cane, affected with ring spot, was growing in the immediate vicinity; consequently the disease affecting Napier grass will be referred to only as an eye spot.

Since the symptoms of the disease as it occurs on sugar cane, and the morphology and physiology of the causal organism have been described by

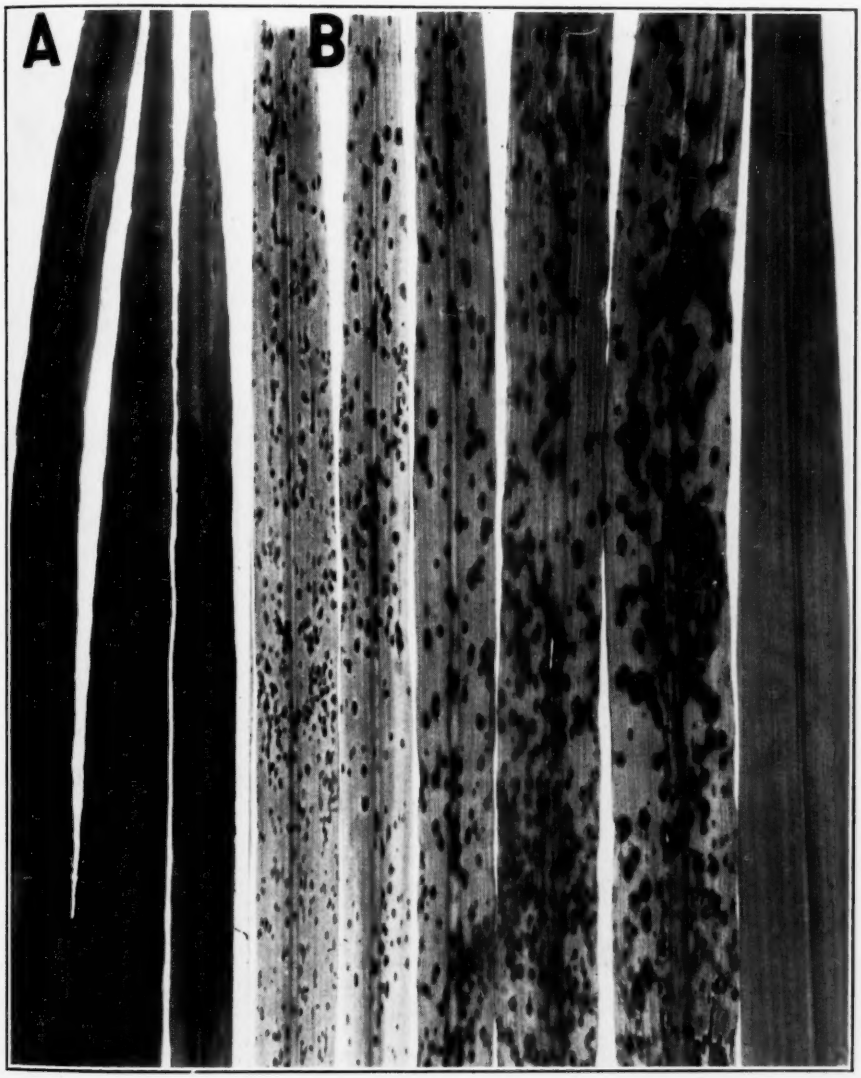

FIG. 1. Typical eye spots on leaves of Napier grass. A. Artificially inoculated in the greenhouse B. Left, naturally infected leaves, showing different stages in the development of eye spots; right, leaf from a resistant plant.

Bourne,[5] this paper deals only with a description of the disease as it occurs on Napier grass, the proof that the causal organism is *Helminthosporium ocellum* and a report of the results of inoculation tests conducted in the greenhouse.

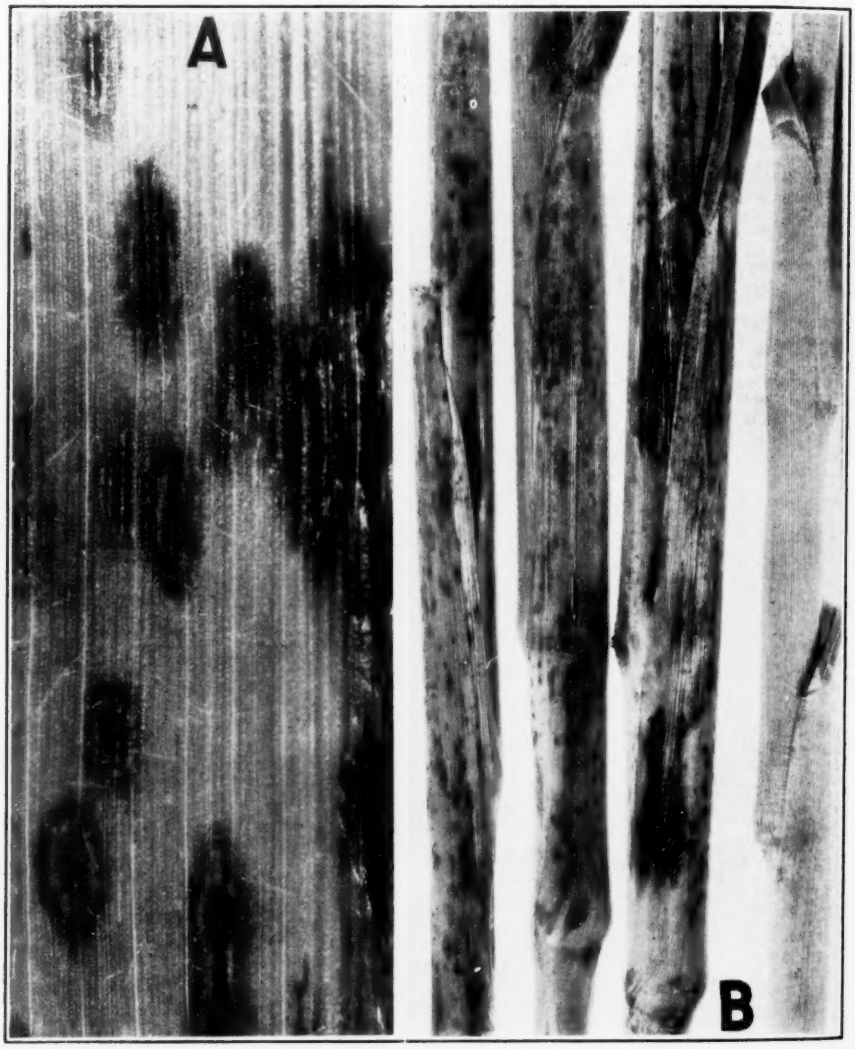

FIG. 2. A. Portion of a Napier grass leaf showing eye spots enlarged. ×7. B. Left, typical spots on leaf sheaths; right, stalk from a resistant plant.

DESCRIPTION OF THE DISEASE

The young spots on the leaves first appear as small, reddish-brown, somewhat oval flecks. On aging they enlarge, the center becomes lighter brown, and the margins a deeper red, until, finally, the centers are of a dirty straw

[5] See footnote 4.

color and the margins a Bordeaux red. The red color predominates in the older spots and the leaves usually assume a reddish cast. By transmitted light, the older spots (Fig. 2, A) usually show a dark fleck in the center surrounded by one or two alternating light and dark areas. The older spots vary in size from 1.5–3 mm. wide by 2–5 mm. long, the greatest dimension being always in the direction of the long axis of the leaf. The spots (Fig. 1, A) tend to remain somewhat oval and regular in outline, except where two or more coalesce and the margins that come in contact disappear, leaving a blotch of irregular outline. The spots on the leaves of some strains of Napier grass develop into elongated streaks similar to those on sugar cane leaves. In cases of heavy infection the leaves wither and die prematurely, the basal leaves first, and drop to the ground.

On very susceptible strains of this grass, the leaf sheaths and stems also

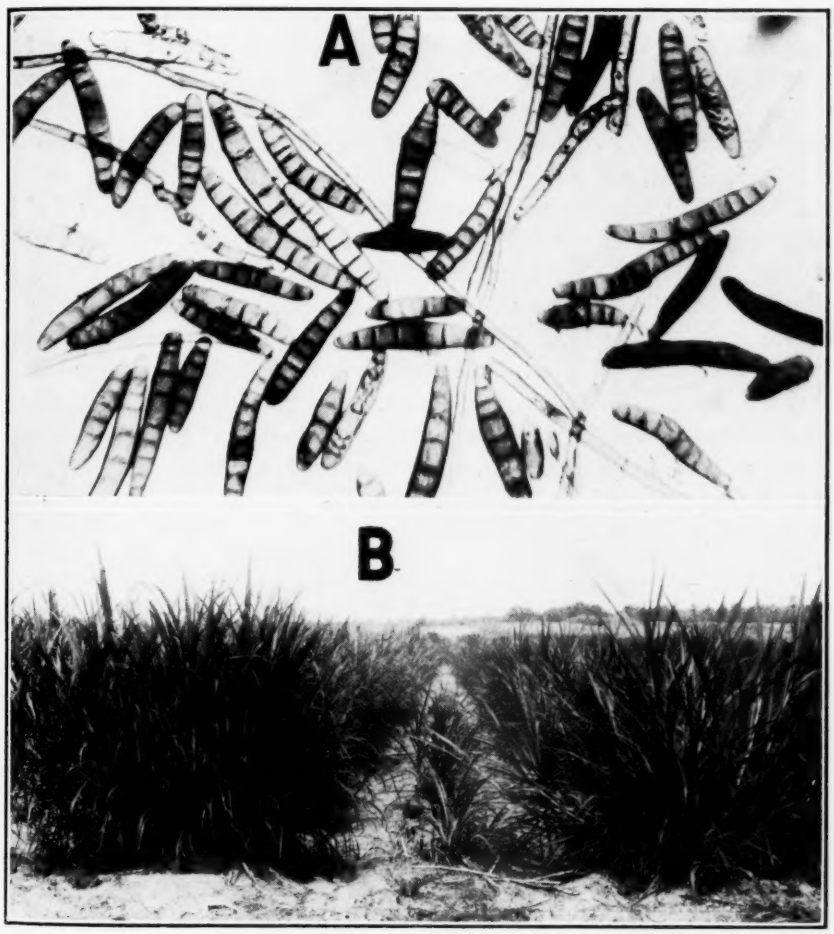

FIG. 3. A. Typical spores of *Helminthosporium ocellum*. ×400. B. View of Napier grass plots showing plants of resistant strains in two outside rows and plants of susceptible strains in center row. Selections made by G. E. Ritchey.

may become heavily infected. The young spots on these parts are similar to those on the leaves, except that a brown rather than red color predominates in all stages in the development of the spots. As the spots become older the contrast in color of the center and margin is much less conspicuous than on the leaves; the entire spot tends to remain brown, the color becoming diluted toward the edges of the spot. The spots (Fig. 2, B) do not become so large as those on the leaves and tend to coalesce and form large, brown, sunken areas.

INOCULATIONS

Young Napier grass plants from root cuttings and seed of resistant and susceptible plants, field-selected by G. E. Ritchey[6] (Fig. 3, B), were grown in the greenhouse and inoculated with a water suspension of spores from pure cultures of *Helminthosporium ocellum* growing on potato-dextrose agar. None of the plants from stock that was immune from the disease in the field became infected in the greenhouse, but all of those originating from susceptible stock developed eye spot in varying degrees, except the noninoculated checks; thus demonstrating that selection of strains of Napier grass naturally immune from or susceptible to the disease can be done effectively in the greenhouse.

A few, small, water-soaked flecks appeared on some of the leaves of the susceptible plants 12 hours after inoculation, but the majority did not appear until after 24 hours. The fresh spots that developed from the flecks closely resembled those that appear on leaves in the field, but as they became older they were surrounded by a distinct halo (Fig. 1, A), which is not so evident on naturally infected leaves. Although *Helminthosporium ocellum* was the only organism isolated from eye spots of Napier grass leaves in both the field and greenhouse, the complete range of morphological characteristics of the disease, as it occurs in the field, was not reproduced by inoculation in the greenhouse, probably because of differences in the environmental conditions of field and greenhouse.

TABLE 1.—*Comparison of spore measurements and number of septa of Helminthosporium ocellum on sugar cane and Napier grass*

Author and host	Length	Average	Width	Average	Number of septa	Average
Faris, Cuba—on sugar cane	29–84 μ	68.9 μ	9–21 μ	12.7 μ	3–10	6.7
Bourne, Florida—on sugar cane	24.1–92.5 μ	58.7 μ	11.1–16.7 μ	14.0 μ	3–9	6.7
Voorhees, Florida—on Napier grass	28–90 μ	56.4 μ	8–14 μ	11.1 μ	3–9	6.5

[6] Associate Agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, and the Florida Agricultural Experiment Station.

THE CAUSAL ORGANISM

The fungus isolated from eye spot of naturally infected leaves of Napier grass was grown in culture and its morphological characters studied. When 200 spores of the fungus were measured, the length, width, and number of septa agreed very closely with the dimensions of *Helminthosporium ocellum* as reported by Faris[7] and Bourne[8] (Table 1). Other characters, including color and type of mycelial growth in culture, color of spores and conodiophores, marked bulbous base of conidiophores, and slight curvature of mature spores (Fig. 3, A), also agree with descriptions given for *Helminthosporium ocellum* and it is concluded that the fungus causing eye spot of Napier grass is the same as that causing the eye or ring spot of sugar cane.

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PHYTOPATHOLOGICAL NOTES

Bacterial Wilt of Sweet Corn in Mexico.—Bacterial wilt of sweet corn has been reported from time to time from most of the southern United States.[1] The disease occurs in the Carolinas, Georgia, Tennessee, Arkansas, and Oklahoma and has been found also in the southern border States of Mississippi, Texas, and New Mexico. The corn flea beetle, *Chaetocnema pulicaria,* has been "reported from most of the United States except the Northwest." "This species was collected by airplane during nearly every month throughout the year at Tallulah" [La.].[2]

Outside of the United States, bacterial wilt has been reported from Puerto Rico and Italy.

While on a vacation trip in Mexico, in December, 1937, green corn was found growing in the lower, more tropical sections in the vicinity of Orizaba, Oaxaca, and Jalapa. At Orizaba and Jalapa, what appeared to be bacterial-wilt lesions were found on the leaves. These lesions were local and typical of the type of lesion commonly occurring on more or less resistant field corn. On returning to Washington, D. C., sections of some of these lesions were examined under the microscope, and bacteria streamed out from the cut ends of fibrovascular bundles. Yellow bacterial colonies developed on plates poured from the lesions on the specimens collected at Orizaba. Pure cultures transferred from these colonies produced typical wilt symptoms on young sweet-corn plants in the greenhouse at Arlington Farm, Virginia. Pure cultures of a yellow organism reisolated from these young sweet-corn plants have again produced typical wilt symptoms on young sweet-corn plants. In sev-

[7] See footnote 3.
[8] See footnote 4.
[1] Rand, F. V., and Cash, L. C. Bacterial Wilt of Corn. Tech. Bul. 362, U. S. D. A., revised, 1937.
[2] Poos, F. W., and Elliott, C. Certain Insect Vectors of *Aplanobacter stewarti.* Jour. Agr. Res. [U. S.] **52**: 585–608. 1936.

eral cases the cultures were sufficiently virulent to kill the inoculated plants. The growth of these pure, yellow cultures on beef peptone agar and beef peptone broth is typical of *Aplanobacter stewarti* (E.F.S.) McC. and, since the lesions produced on corn are typical of bacterial wilt, there can be no question of the occurrence of this disease in Mexico. While corn appears to be grown in all sections of Mexico, even up to 9,000 feet, or higher, on the mountain slopes, in December all of the corn on the plateaus and mountain slopes was mature and much of it harvested. Consequently, it was not possible to make observations of the abundance and distribution of the disease in Mexico at that time. All of the teosinte plants found were also mature and dry and no information was obtained on the occurrence of the disease on this natural host, which is native in this area.

On the green corn plants infected with wilt in the vicinity of Orizaba, insect feeding injuries similar to those caused by *Chaetocnema pulicaria* were observed on the leaves and some of the smaller lesions appeared to be developing from these feeding injuries. A few small flea beetles, which appeared to be *Chaetocnema pulicaria,* were collected from these corn plants and were submitted for identification to H. S. Barber, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, who found the single included male, from Orizaba, to agree in all external details as well as in the peculiar shape of the aedeagus with those of *C. pulicaria* from the eastern United States and who believed the several female specimens from Orizaba and Oaxaca to belong also to that species. Apparently, therefore, *C. pulicaria* occurs in the vicinity of Orizaba and Oaxaca, Mexico, and probably serves as a vector for the bacterial-wilt organism there, as well as in the United States. No isolations were made from these beetles collected in Mexico.—CHARLOTTE ELLIOTT, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Observations on the dissemination of fungi by ants.[1]—Bailey[2] in 1920 presented a comprehensive discussion of "Some relations between ants and fungi" in which he showed that the infrabuccal pellets of many ants often contain large quantities of fungus spores and mycelium. He states that "It is the general tendency among ants to take spores, fragments of mycelia, and bits of decaying plant tissue into their infrabuccal pockets. In this sac the fungi become mixed with food residues and mineral and vegetable detritus, forming a pellet which is subsequently voided upon any convenient surface." The composition of the infrabuccal pellet suggests that it is a "culture or potential source of infection." He pictured the contents of several pellets in which could be recognized the spores of many fungi belonging to genera that include important plant pathogens. His studies were made on specimens preserved in alcohol, and the viability of spores in fresh

[1] Paper Number 1578 of the scientific journal series, Minnesota Agricultural Experiment Station.

[2] Bailey, I. G. Some relations between ants and fungi. Ecology **1**: 174–189. 1920.

pellets was not tested. Bailey pointed out the "necessity for further and more detailed investigations upon the structure and feeding habits of the Formicidae and the role of these insects in the dissemination of plant diseases."

Whetzel,[3] in a discussion of Botrytis blight of peony, stated, "Ants seem to carry the spores from the base of diseased stalks to the buds of healthy plants. Here, in the exuded sugary solution, so abundant upon the unopened peony buds, the spores find both food and moisture and germinate much more promptly and vigorously than in water." No mention was made of the method in which the spores are carried by the ants.

The writers have observed ants feeding on the sugary secretion of young peony buds, many of which became necrotic and failed to open. Attempts were made to determine whether the ants were distributing fungus spores in their infrabuccal pockets and, if so, whether the spores were retaining their viability and causing infection. Careful observations by the writers revealed the presence on the buds and stems of many black pellets, later identified as voided infrabuccal pellets. Ants (*Formica fusca* var. *subsericea* Say) were taken from peony buds and the infrabuccal pellets dissected out and examined microscopically. Spores of several different kinds of fungi were found, the most prevalent being *Alternaria*. When the pellets were crushed in hanging drops of sterile distilled water, many of the spores germinated (Fig. 1), showing that viable spores may be disseminated in this way.

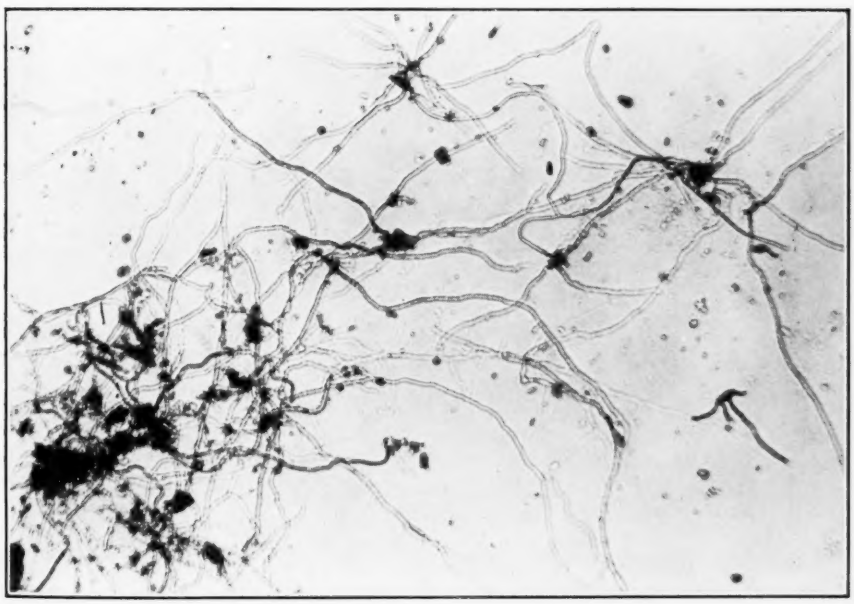

FIG. 1. Mycelium arising from viable spores of *Alternaria* sp. from an infrabuccal pellet of *Formica fusca* crushed in a drop of sterile distilled water.

[3] Whetzel, H. H. Diseases of the peony. American Florist **44**: 609–612. 1915.

It was not possible to show any relationship between the fungus spores disseminated by the ants and the blighting of the peony buds. Necrotic petals taken from buds in early stages of necrosis proved to be sterile, indicating that the blight probably was not caused by a parasite. In later stages of development a species of *Alternaria* and an unidentified white hyphomycete were isolated. When young peony buds were sprayed with spore suspensions of these fungi, however, they did not become blighted. Also, when 4 peony plants were protected from ants by means of a metal cylinder and a band of "tanglefoot" glue, the amount of bud blight was not reduced significantly.

These observations show that viable fungus spores are disseminated in the infrabuccal pellet of ants but that the ants associated with peony buds in these experiments were not involved in the development of the bud blight. Peony blights caused by *Botrytis* and *Phytophthora* were not prevalent when these experiments were made, and no spores of these fungi were found in the infrabuccal pellets of the ants or on the blighted buds. Since ants, in general, do not make wounds in plant tissue the likelihood of ants acting as effective vectors of plant diseases is probably less than that of insects that puncture the tissue. Ants that visit flowers or fresh wounds, however, should be looked upon with suspicion.—J. G. LEACH and LOUISE DOSDALL, University Farm, St. Paul, Minnesota. Cooperative investigations between the Divisions of Plant Pathology and Entomology, University of Minnesota.